From Programmatic Goals to Criteria for Phytoplankton Chlorophyll *a*

A report prepared by

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Executive Summary

Virginia Department of Environmental Quality (VADEQ) initiated the James River Chlorophyll Criteria Study in 2011 to review the scientific basis of the numeric chlorophyll *a* criteria applied to James River tidal waters. The Study uses mostly recent data and results from the tidal James River to evaluate protectiveness of the criteria. This report is not the Study's final report. Rather it summarizes some longer-term analyses and broader findings that have relevance to Chesapeake Bay as a whole, including the tidal James River. Although the author is a member of the Study's Scientific Advisory Panel (SAP), the conclusions in this report are the author's and do not necessarily reflect the opinions of other panel members.

The report derives, in a logical progression, numeric chlorophyll *a* thresholds that are protective of Chesapeake Bay's designated uses. The progression begins with programmatic goals found in Chesapeake Bay Program agreements and state water quality standards, and builds on the narrative standards, principal ecological concepts, and empirical evidence summarized here:

I. **Programmatic goals** for nutrients and water clarity reflect society's expectations for a restored Chesapeake Bay, including the tidal James River in Virginia.

II. **Numeric ranges for nutrients and water clarity** that achieve the narrative water quality goals are found in Chesapeake Bay research results, data analysis, and historical accounts.

III. Multi-metric **indices of biotic integrity for phytoplankton** can be developed from populations currently inhabiting waters that meet the narrative water quality goals. These "reference" populations have many ecologically desirable characteristics.

IV. **"Balanced, indigenous, desirable" aquatic life** is a designated use of tidal waters in Virginia water quality standards.[1] Designated uses are regulatory goals. At this time, multi-metric reference-based indices of biotic integrity best represent phytoplankton populations that are achieving and supporting the aquatic life designated use.

V. **Chlorophyll *a*** is an indicator of phytoplankton biomass and statistical properties of large chlorophyll *a* data sets are useful in evaluating biotic integrity of phytoplankton populations.

VI. **Deleterious algal blooms** are associated with frequent high concentrations of chlorophyll *a* and poor phytoplankton biotic integrity. Blooms are stimulated by excess nutrients and are associated with immediate and long-term negative impacts on estuarine aquatic life. They also impinge on two other designated uses: recreation (swimming, boating) and production of edible, marketable natural resources (fish, shellfish).

VII. Narrative **chlorophyll *a* criteria** in Virginia's state-wide water quality standards call for protection *of* aquatic life that meets designated uses as well as protection *against* the deleterious impacts of algal blooms. Data analyses are identifying a range of chlorophyll *a* thresholds protective *of* balanced desirable populations at lower concentrations and protective *against* algal bloom impacts at higher concentrations.

VIII. **Choice of a chlorophyll a criteria statistic** can be flexible due to the inherent properties of large chlorophyll *a* data sets. A data set's central tendency (mean, median, geometric mean) is closely

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[1] 9 VAC 25-260-10. Designated Uses: The propagation and growth of a balanced, indigenous population of aquatic life, including game fish, which might reasonably be expected to inhabit [Virginia waters]. 9 VAC 25 260 20. General Criteria: Specific substances to be controlled include …. substances which nourish undesirable or nuisance aquatic plant life.

Table ES - 1. Current James River chlorophyll *a* criteria (µg/liter), which are seasonal geometric means, and their projected upper limits (90th percentiles). Over time, ten percent of chlorophyll *a* values in an assessment unit can be expected to occur above the upper limit. These criteria are not protective of many designated uses.

	Criteria	Projected Upper Limit
Spring Tidal Fresh	10[1], 15[2]	27[1], 40[2]
Spring Oligohaline	15	40
Spring Mesohaline	12	33
Spring Polyhaline	12	33
Summer Tidal Fresh	15[1], 23[2]	29[1], 45[2]
Summer Oligohaline	22	43
Summer Mesohaline	10	20
Summer Polyhaline	10	20

[1] upper tidal fresh segment; [2] lower tidal fresh segment

associated with the magnitude of its highest concentrations and the frequency of exceeding a threshold or criteria. A criteria statistic can be selected to suit the type of data used in state assessments (e.g., shipboard, continuous monitoring, DATAFLOW).

The current chlorophyll *a* criteria for the tidal James River are applied as the seasonal geometric mean of chlorophyll *a* measurements in a salinity-based assessment unit. In long-term Chesapeake Bay monitoring data, the natural variability of chlorophyll *a* values around each criterion's geometric mean indicates an expected upper limit associated with that mean (**Table ES-1**). Protectiveness of the James River chlorophyll *a* criteria can be evaluated with either the geometric means or their projected upper limits.

The current criteria are not protective of phytoplankton reference populations, which are the best available representations of balanced, indigenous, desirable phytoplankton life in Chesapeake Bay. They do not appear protective of baywide water clarity requirements for healthy submerged aquatic vegetation in near-shore waters. Baywide analysis shows the criteria may not be protective against potential harm from the toxin producing *Microcystis* and *Prorocentrum*. The criteria may be somewhat protective of the dissolved oxygen requirements for healthy benthic macroinvertebrate populations in deep waters of the Bay mainstem. The criteria are not protective of four of the eight season- and segment-based "defensible ranges" proposed in the ongoing James River Chlorophyll *a* Criteria Study, but may be partially protective of the four other "defensible ranges."

Making Virginia's already stringent assessment methodology more stringent is not likely to make the chlorophyll *a* criteria fully protective of all designated uses. If Virginia decides to continue to rely on the geometric mean as the criteria statistic, one option to make the criteria more protective would be to lower their numeric values. Another option would be to keep the same numeric values and change the criteria statistic from a geometric mean to an upper limit. Both options involve changing Virginia's established water quality standards, and a reexamination of Virginia's current assessment methodology would then be warranted. Multiple assessment methodologies could be needed if high-frequency data types (DATAFLOW, continuous monitoring sondes, satellite imagery) are used in future assessments.

Analysis of Chesapeake monitoring data shows that chlorophyll *a* criteria alone will not protect phytoplankton reference populations. Very poor water clarity caused by suspended sediments and other non-living matter impedes phytoplankton photosynthesis and growth. Chlorophyll *a* concentrations are kept low and can falsely indicate criteria attainment. Cells are physiologically stressed and facultative and motile taxa are favored, including several known toxin producers. If water clarity screening thresholds or criteria are also applied to tidal open waters, attainment of both water clarity and chlorophyll *a* criteria will be protective of phytoplankton reference communities and, by extension, Virginia's aquatic life designated uses for tidal waters.

From Programmatic Goals to Criteria for Phytoplankton Chlorophyll *a*

The tidal James River chlorophyll *a* criteria are the immediate subject of this report[2] but the river's criteria are considered in the context of the larger Chesapeake Bay and its tidal tributaries. A logical progression of steps connects programmatic water quality goals to chlorophyll *a* endpoints protective of Chesapeake Bay's aquatic life designated uses. The steps build on existing water quality standards, principal ecological concepts, and empirical evidence from the Bay's tidal waters. This report is not the official Scientific Advisory Panel (SAP) report for the James River Chlorophyll *a* Criteria Study. Rather it summarizes some longer-term analyses and broader findings that have relevance to Chesapeake Bay as a whole, including the tidal James River.

Chlorophyll *a* is a light-sensitive chemical essential for photosynthesis in plants and algae. It correlates strongly with phytoplankton biomass in estuarine open water environments and high concentrations are a well-known indicator of nutrient enrichment. Recognizing the chemical's usefulness as an indicator, the Chesapeake Bay Program (CBP) developed narrative chlorophyll *a* criteria (USEPA 2003) for Bay tidal waters which have since been adopted into the water quality standards of Maryland, Virginia, and the District of Columbia. Virginia implemented numeric chlorophyll *a* criteria for the tidal James River because algal-related impairments were expected to remain there after dissolved oxygen and water clarity criteria were attained (VADEQ 2004).

Open water environments of the tidal James River are eutrophic, with high nutrient and sediment inputs and frequent algal blooms. Phytoplankton community composition is unbalanced and levels of undesirable algal taxa are increasing. The tidal river was listed as impaired in 1999 for violation of Virginia's Water Quality Standards and included in the USEPA's Chesapeake Bay TMDL issued in 2010. The Bay TMDL, described as a "pollution diet," is intended to bring into baywide compliance all tidal water quality standards. In 2011, for reasons relating to the Chesapeake Bay TMDL Phase I Watershed implementation Plan (WIP), Virginia Department of Environmental Quality (VADEQ) initiated the James River Chlorophyll *a* Criteria Study to review the river's numeric chlorophyll *a* criteria and determine the best scientific basis for the standard.

I. Programmatic Goals

Two narrative restoration goals concerning the chemical and physical properties of tidal open water environments are often expressed in regional, Chesapeake Bay Program (CBP) agreements and technical documents. They reflect to a large extent society's expectations of restored water quality in Chesapeake Bay, which includes the tidal James River. The goals are:

- Concentrations of the nutrients nitrogen and phosphorus that limit the formation of algal blooms (aimed primarily at reducing deep water anoxia in summer)
- Water clarity adequate for normal photosynthesis by aquatic plants (aimed primarily at restoring submerged aquatic vegetation, or SAV)

[2] The basis of this report was a "white paper" (Buchanan 2015b) responding to a VADEQ request for feedback on preliminary results of the James River Chlorophyll *a* Criteria Study and ongoing discussions of the study's Scientific Advisory Panel (SAP). The white paper drew on information available at the time through the SAP, including unpublished analyses, SAP presentations, agency reports and documents, and published papers. This report restructures the white paper and includes additional data analysis results.

Open water environments achieving these two goals, herein called "reference conditions," exemplify desirable water quality conditions. They are thought to allow primary producers at the base of the aquatic food web to grow normally and support a heathy Chesapeake Bay ecosystem and all of its designated uses. This perception is based on available science and historical accounts of Chesapeake Bay. A similar goal is expressed in the Virginia Water Quality Standards Regulation, which requires that "substances nourishing undesirable or nuisance aquatic plant life" be controlled (9 VAC 25-260-20). The nutrient reductions set in the Chesapeake Bay TMDL are intended to achieve these desired endpoints.

Programmatic goals also exist for water column chlorophyll *a*, a proxy for phytoplankton biomass. Chlorophyll *a* is sometimes viewed as a chemical property of water itself. It is more correctly thought of as a light-sensitive molecule critical to the survival and photosynthetic functions of phytoplankton and underwater grasses, the two major primary producers in Chesapeake Bay food webs. Thus, goals for water column chlorophyll *a* are describing the desired phytoplankton responses to good water quality.

II. Numeric Ranges for Nutrients and Water Clarity

Factors that most strongly govern Chesapeake Bay phytoplankton populations are season (temperature, incident light, day length), salinity, mixing (flow, residence time, stratification), light attenuation (water clarity) and concentrations of bioavailable nitrogen and phosphorous. The first three factors are not controllable. To account for the effects of these natural factors on phytoplankton, researchers and analysts typically parse experimental and empirical data into groups defined by season, salinity, and water column layer. The remaining parameters are strongly influenced by anthropogenic activities and thus are to some extent controllable.

Nutrient bioassay studies (e.g., Fisher & Gustafson 2003, L. Haas, others), a data analysis approach called the Relative Status Method (see Olson 2002, 2009), historical data sets, and literature reviews provide scientific information about the minimum light levels and maximum dissolved nutrient concentrations meeting the two programmatic water quality goals above. The light and nutrient thresholds characterizing reference conditions for phytoplankton are in general agreement with those for underwater grasses, or submerged aquatic vegetation (SAV). Information about reference conditions for SAV is synthesized in Batiuk *et al.* (1992, 2000) and consists of minimum levels for water clarity and maximum levels for phytoplankton chlorophyll *a*, dissolved inorganic nitrogen (DIN), ortho-phosphate (PO_4), and total suspended sediments. Ambient water quality that achieves reference conditions for either of these primary producers will end up benefiting both.

Characterizations of aquatic habitats are most informative when the controllable factors are considered together, using a binning approach, rather than separately. This is because an organism's growth at any one time is controlled by the scarcest of its resources (limiting factor), not by the total amount of each resource available, and when one factor ceases to be limiting, another becomes limiting (Liebig's "law of the minimum"). Bins are used to represent distinct, multi-dimensional environments, e.g., two factors are not limiting and a third one is limiting. They provide a more holistic view of an algal cell's actual environment and suggest management approaches that differ from the more 'linear' approaches based on an organism's response to a single condition or pollutant (e.g. stressor-response models).

Approaches for Characterizing Reference Water Quality Conditions

The two analytical approaches below best identified nutrient and light ranges that sustain desirable populations of tidal phytoplankton. To date, a multi-metric water quality index has not been developed for phytoplankton habitat although such an index could be developed.

1. Water quality benchmarks

With support from the CBP Tidal Monitoring and Analysis Workgroup (TMAW), Olson (2002) analyzed historical and CBP data sets with a Relative Status Method and established benchmarks for total phosphorus (TP), total nitrogen (TN), suspended solids (TSS), and chlorophyll *a* concentrations. CBP segments with median concentrations of TP, TN, TSS and chlorophyll *a* all in the desirable (lower 1/3) ends of their total ranges were identified as "Good." Upper percentiles of the *entire* distributions of TP, TN, TSS and chlorophyll *a* in these Good segments were then used to identify the benchmarks. Benchmark results for the 1950s to 1980s decades, when Chesapeake Bay was considered relatively heathy, characterize reference conditions for phytoplankton. The benchmarks were used for a time by Maryland and Virginia to report baywide status and trend results.

Strengths:

- TSS concentrations in the historical reference locations are substantially lower than post-1990s levels and meet SAV habitat requirements in mesohaline and polyhaline salinities, indicating Secchi depths were much deeper historically.
- Mean, median, and the 10th and 90th percentiles for TP, TN, TSS, and chlorophyll *a* are calculated for five decades and multiple CBP segments, including the James River, so variation within decades and locations can be determined.
- The overall benchmarks are derived from relatively large sample sizes, which increases confidence in the results and the suggested relationships between nutrients and light.
- The benchmarks agree with literature values for mesotrophic conditions (See Table 2a-d in Olson 2002, review in USEPA 2003, 2007b).

Weakness:

- TP and TN are composite nutrient metrics which contain phytoplankton N and P, thus TN and TP benchmarks are confounded by the phytoplankton component.
- TSS is not the only parameter presently attenuating light in Chesapeake Bay; historical light attenuation by Colored Dissolved Organic Matter (CDOM) is not available.
- Water quality degradation was occurring during the historic period (1950s – 1980s) so benchmarks from that era do not reflect least-degraded conditions in some Bay areas.
- The historic data were collected primarily in the Bay mainstem and lower tributaries.
- The benchmarks were not created with phytoplankton habitat requirements in mind.

2. Water quality categories characterizing phytoplankton Reference conditions

With further support from CBP, results of the Fisher and Gustafson (2002) bioassay experiments and another Relative Status Method analysis were used to identify season- and salinity-specific water quality categories for phytoplankton (USEPA 2003, Buchanan *et al.* 2005, Olson 2009). Conditions meeting all three thresholds achieve the two water quality programmatic goals above and were classified as Reference (REF). Conditions failing all three thresholds are classified as Degraded (DEG).[3] Intermediate conditions were also identified. Conditions with adequate light and one or both nutrients in excess amounts were classified as Mixed Better Light (MBL). Conditions with inadequate light and one or both nutrients low enough to limit bloom formation were classified as Mixed Poor Light (MPL). Reference and Mixed Better Light conditions, identified as REF+MBL, were sometimes analyzed together. Phytoplankton populations in MBL resemble those in REF in many regards (more below) and the MBL category was useful as a surrogate REF.

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[3] From here on, the capitalized terms "Reference" and "Degraded" refer to the water quality categories developed for Chesapeake Bay phytoplankton with the Secchi depth, DIN, and PO_4 thresholds described in Buchanan *et al.* (2005) and refined in Buchanan (2015a), and to the phytoplankton communities in those water quality conditions

Strengths:

- The water quality categories can be useful in a prediction capacity.
- Reference conditions are supportive of CBP and Virginia nutrient and water clarity programmatic goals.
- The different combinations of limiting and non-limiting Secchi depth, DIN, and PO_4 expressed in the water quality categories (bins) correspond to significant differences in phytoplankton community-level metrics and taxonomic composition.
- Further dividing the nutrient and light classes produces new, higher resolution categories of water quality with greater predictive capability.

Weaknesses:

- The use of bins sometimes makes it difficult for analysts to identify which of the three water quality parameters – light, DIN, or PO_4 – exerts the greatest influence on phytoplankton.
- Reference conditions occur often in high mesohaline and polyhaline salinities, but occur much less often in tidal fresh or oligohaline salinities. The sparseness of Reference conditions in the tidal fresh and oligohaline makes is necessary to use the MBL as a surrogate for Reference.

Several other multi-metric habitat indices exist for Chesapeake Bay but they are not tailored to phytoplankton habitat requirements. Williams *et al.* (2009) developed an index of overall Chesapeake Bay health composed of a water quality index based on chlorophyll *a*, dissolved oxygen, and Secchi depth scores, and a biological index of lower trophic levels consisting of the phytoplankton (PIBI) and benthic macroinvertebrate (BIBI) index scores, and percent attainment of CBP's SAV goal. CBP recently developed a Water Quality Standards Achievement index based on chlorophyll *a*, dissolved oxygen, and Secchi depth, and uses that index to track progress meeting Bay water quality standards. Bay resource agencies and organizations use SAV habitat requirements (water quality thresholds) to identify potential sites for SAV restoration.

Water Quality Screening Thresholds

The nutrient thresholds for phytoplankton Reference conditions ($\leq$ 0.07 DIN mg/liter, $\leq$ 0.007 PO_4 mg/liter) were determined in carefully controlled nutrient bioassay experiments with additions of DIN and PO_4 (Fisher and Gustafson 2003). However, the thresholds probably should not be used as nutrient screening thresholds or criteria for open water environments. Nitrogen and phosphorus are "substitutable resources," meaning algae and plants can obtain these essential elements from other, less bioavailable compounds (e.g. Wetzel 2001, Lampert and Sommers 1997). For example, algae and plants are able to obtain nitrogen from organic nitrogen compounds to varying degrees. Algae and plants also have a well-recognized capacity to store phosphorus, so ambient concentrations are usually lower than intra-cellular concentrations. Light energy, on the other hand, is a "non-substitutable resource."

Secchi depth thresholds identified for Reference conditions could be used as water clarity screening thresholds or even criteria in open waters environments. Although first determined with the Relative Status Method, the thresholds were later found to correspond well with the onset of photosynthetic stress in phytoplankton, namely an increase in chlorophyll cell content (Chl:C ratio). Higher Chl:C ratios indicate the average cell in the above-pycnocline layer is spending more time below its "compensation" depth (usually 0.1%-1.0% light penetration depth), in light levels inadequate for normal photosynthesis. To compensate, cells increase their chlorophyll cell content and capture more of the fewer light photons. If the compensation depth becomes too shallow, taxa such as facultative autotrophs (e.g. dinoflagellates) or those with buoyancy mechanisms (e.g. blue-greens) become more competitive.

The proportion of time an average cell spends in adequately lit waters is governed largely by water clarity and the depth of the surface mixing layer in an estuary. Chesapeake Bay's relatively shallow and irregular bathymetry, it's partially mixed circulation, and the variable influences of season and freshwater flow on stratification constantly modify the extent to which phytoplankton mix vertically. Thus, the proportion of time an average phytoplankton cell spends in adequately lit waters is always changing. Secchi depth thresholds for phytoplankton Reference conditions can be considered generalized targets for Chesapeake Bay water clarity.

Secchi depth thresholds that characterize the water clarity requirements for SAV are roughly the same as those for phytoplankton in tidal fresh and oligohaline salinities, but are less stringent in mesohaline and polyhaline salinities (**Table 1**). Attainment of the SAV-based water clarity criteria in both near-shore and open waters should protect against algal blooms in low salinities and be somewhat protective against algal blooms in high salinities.

Table 1. Thresholds of adequate water clarity for phytoplankton in open water habitats and forsubmerged aquatic vegetation (SAV) in nearshore habitats, expressed as Secchi depth, in meters. Sources: * Buchanan *et al.* (2005) for phytoplankton Reference communities; ** Buchanan (2015a) for phytoplankton Reference communities, *** Batiuk *et al.* (1992) from SAV Technical Synthesis I (Secchi depth = 1.45/kd); *** Batiuk *et al.* (2000) from SAV Technical Synthesis II (Secchi depth = 1.45/kd).

Season/	Salinity Zone	Phytoplankton Thresholds *	Phytoplankton Thresholds**	SAV Restoration to 1 Meter ***	PLW Secondary Requirement ****
Spring	TF	>0.9	>0.8	>0.725	>0.711 (PLW=13%)
	OH	>0.7	>0.8	>0.725	>0.711 (PLW=13%)
	MH	>1.8	>1.4 (LoMH) >1.8 (HiMH)	>0.967	>0.958 (PLW=22%)
	PH	>2.15	>2.1	>0.967	>0.958 (PLW=22%)
Summer	TF	>0.8	>0.8	>0.725	>0.711 (PLW=13%)
	OH	>0.6	>0.8	>0.725	>0.711 (PLW=13%)
	MH	>1.45	>1.2 (LoMH) >1.6 (HiMH)	>0.967	>0.958 (PLW=22%)
	PH	>1.85	>1.8	>0.967	>0.958 (PLW=22%)

III. Indices of Biotic Integrity for Phytoplankton

Clear differences between biological populations in "good" and "bad" habitat conditions are evidence that the environmental parameters selected to characterize those habitat conditions significantly influence the biota (Martinez-Crego *et al.* 2010). Clear differences in multiple, community-level metrics have been documented for phytoplankton populations in Chesapeake Bay's Reference and Degraded water quality conditions (Buchanan *et al.* 2005, Marshall *et al.* 2006, Lacouture *et al.* 2006, Johnson and Buchanan 2013). The most responsive are blue-green biomass, dinoflagellate biomass, diatom biomass, total biomass, % cryptophyte biomass, pico-phytoplankton abundance, pheophytin, dissolved organic carbon (DOC), C:Chla ratio, and chlorophyll *a*. Taxonomic differences have also been documented in Virginia's high salinity (>10‰) waters, where numbers of samples collected from Reference water quality conditions are sufficient (Buchanan 2015a). About one third (170) of the observed taxa or taxonomic groups appear often enough in phytoplankton sample counts to serve as potential indicator taxa. A diverse set of forty-five (45) taxa or taxonomic groups appear more frequently and/or in higher overall or maximum abundances in Degraded conditions. They include known or suspected toxin producers or nuisance bloom formers (HABs). Another thirty-two (32) taxa or taxonomic groups, mostly diatoms, appear more frequently and/or in higher overall or maximum abundances in Reference conditions. One is a known toxin producer, but toxic strains of this taxa are not found in the Mid-Atlantic region. Metrics derived from High Pressure Liquid Chromatography (HPLC) data have the potential to characterize phytoplankton taxonomic composition in different water quality conditions, but this application of the data has not been explored.

Individual metrics based on community and taxonomic features can be sensitive measures of phytoplankton responses to specific stressors (e.g. response of intra-cellular chlorophyll *a* content, or Chl:C ratio, to light). By themselves, however, they are not considered the most appropriate measures of phytoplankton biotic integrity. This includes the popular metric chlorophyll *a*. Indices based on multiple, diverse features of a biological population are generally considered better measures of status and biotic integrity than individual metrics (e.g., National Academy of Sciences 1992, Gibson *et al.* 2000, Simon 2003, Martinez-Crego *et al.* 2010). Indices of this kind for Chesapeake Bay phytoplankton include the Phytoplankton Index of Biotic Integrity (PIBI) and a recently developed Phytoplankton Taxonomic Index (PTI). Both indices distinguish between Reference and Degraded conditions with a high degree of certainty (**Figure 1**).

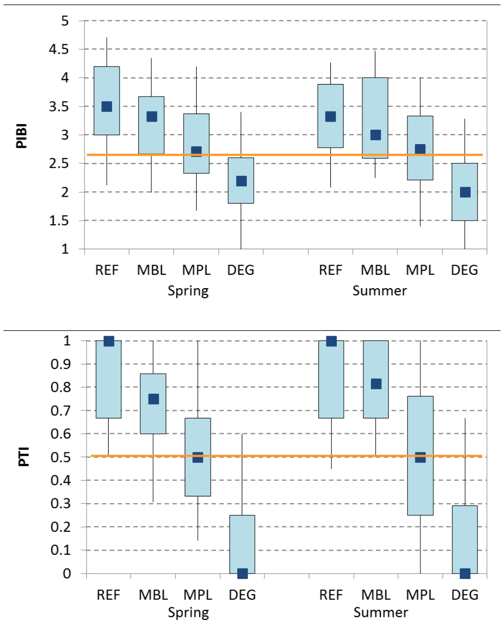

Figure 1. Spring and summer distributions of Phytoplankton Index of Biotic Integrity (PIBI) and Phytoplankton Taxonomic Index (PTI) scores in high salinity waters (>10 ppt), for four water quality categories: REF, Reference; MBL, Mixed Better Light; MPL, Mixed Poor Light; DEG, Degraded (see text for details). Box-and-whiskers indicate 5th, 25th, 50th, 75th and 95th percentiles. Orange line separates acceptable (above) and unacceptable (below) rankings.

Multi-Metric Indices for Chesapeake Bay Phytoplankton

The PIBI index contains community-level metrics representing photosensitivity, total biomass, taxonomic composition, physiological stress, and two potentially harmful taxa. Productivity, a rate metric, would have been a good metric but it was not used because of past differences in the Maryland and Virginia methods. The PTI index contains abundance-based scores of individual family- and genus-level taxa. Scoring for both is based on distributions of metric values in Reference conditions.

Strengths:

- The PIBI's component metrics characterize a variety of phytoplankton community structures and functions, not just one feature.
- The use of metric *scores* rather than actual metric values avoids giving undue weight to component metrics of the index and biasing the overall index.
- The PIBI has broad-scale (baywide) applicability because season- and salinity-specific thresholds are applied.

- The PIBI and PTI are typically better than their individual metrics at distinguishing Reference from Degraded conditions (see below).
- High PIBI and PTI scores are also achieved in intermediate MBL water quality conditions. These populations can be used as surrogates for Reference populations when Reference conditions are sparse.
- The PIBI and PTI both have relatively high classifications efficiencies (see below).

Weaknesses:

- The PIBI could contain more metrics of indicator species (HABs, food quality for grazers).
- The PIBI and PTI are best analyzed over time frames longer than Virginia's 3-year assessment period because the phytoplankton monitoring program has low sampling frequency and poor spatial coverage.
- Bay phytoplankton monitoring programs are, at times, subject to funding gaps and a lack of political commitment.
- The PIBI and PTI are taxonomy-based and thus dependent on an adequate, consistent level of taxonomic expertise in laboratory staff.
- The PIBI and PTI are not designed to detect ecosystem deterioration at an early stage.[4]
- It is difficult to link deteriorating index values with a specific, causative stressor because the index is sensitive to multiple stressors.[4]

Classification Abilities

The ability of individual phytoplankton metrics to correctly identify samples from known Reference and Degraded waters is called the discrimination efficiency (DE). Over the 1984-2013 period, DEs of the individual metrics included in the PIBI index varied widely across the eight season- and salinity-specific habitats, averaging 68.0% and ranging from 52.7% to an unusually high 92.7% (summer tidal fresh DOC). DEs of surface chlorophyll *a* range from 52.7% (spring tidal fresh) to 73.9% (summer mesohaline). The classification efficiency (CE) statistic quantifies the same ability in a multi-metric index. CEs for the season- and salinity-specific PIBI index scores are typically higher than DEs of individual metrics, averaging 76.6% and ranging from 69.7% to 84.4%. The PTI has scoring protocols for 77 phytoplankton taxa. On average, 4.9 (spring) and 4.8 (summer) taxa appear in a sample and have abundances that are high or low enough to trigger the scoring protocol. The PTI index is the average of those scores. CEs for the index are 89.1% in spring and 90.4% in summer. In other words, the PTI index correctly indicates Reference and Degraded conditions about 9 times out of 10. The analyses show that the multi-metric PIBI and PTI indices identify Reference and Degraded water quality conditions better than individual phytoplankton metrics. Therefore, more confidence should be placed in the index scores. The analyses also demonstrate the similarity in index scores from Reference and MBL conditions.

Secchi depth, DIN, and PO_4 appear to adequately characterize phytoplankton habitat conditions. The relatively high CEs of the PIBI and PTI indices indicate phytoplankton are particularly sensitive to water clarity and the nutrients DIN and PO_4. This is seen in the good separation between Reference and Degraded PIBI and PTI scores (**Figure 1**). The three water quality parameters appear to be the major environmental factors controlling phytoplankton communities in Chesapeake Bay outside of season, salinity, and water column mixing and stratification. Management actions that sufficiently improve water clarity and reduce nitrogen and phosphorus concentrations should make desirable changes in the Bay's phytoplankton.

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[4] From review by Martinez-Crego *et al.* (2010)

IV. "Balanced, Indigenous, Desirable" Aquatic Life

Designated uses are regulatory goals in a state's water quality standards. They are intended to protect specific conditions. "The propagation and growth of a balanced, indigenous population of aquatic life … which might reasonably be expected to inhabit [Virginia waters]" is a designated use in Virginia water quality standards and applies to all state waters (9 VAC 25 260 20). To attain this designated use, "…. substances which nourish undesirable or nuisance aquatic plant life" are to be controlled (9 VAC 25-260-10). It is important to note that designated uses apply not to individual parameters such as chlorophyll *a* but to populations of aquatic life and their community structure (taxonomic composition), function, and sustainability.

Phytoplankton populations found in tidal waters that meet narrative nutrient and water clarity goals can be considered achievable reference populations (as opposed to historical reference populations). These Reference populations are the best available representation of "balanced, indigenous, desirable" phytoplankton communities in Chesapeake tidal open water habitats at this time. Characteristics of Reference populations include:

- Relatively stable levels of total biomass with low risk of algal blooms
- Sufficient phytoplankton food for grazers
- Relatively small percentages of cyanophyte (blue-green) and dinoflagellate taxa
- Unstressed photosynthesis, as indicated by consistently low intra-cellular chlorophyll *a* content (Chla:C ratio)
- Less physiological stress, as indicated by low pheophytin and DOC levels
- Larger average cell size
- Rare occurrences and/or low abundances of nuisance/toxic phytoplankton taxa
- Low to moderate chlorophyll *a* levels typical of mesotrophic conditions (*cf.* USEPA 2003)
- Somewhat higher taxa richness (but little or no difference in Shannon-Wiener Diversity or Pielou Evenness) [5]

Rankings

Biological populations in reference conditions can be used as "standards or benchmarks against which to compare the current condition" of other populations (Martinez-Crego *et al.* 2010). The middle range of 2.67 – <3.33 on the PIBI scale of 1.0 – 5.0 has been identified by CBP as the minimal acceptable level of phytoplankton biotic integrity. Scores of 2.67 or higher are considered to be Reference-like; those less than 2.67 represent populations that are least like Reference, or Degraded. This is a common approach for identifying acceptable scores in IBIs derived with a 1-3-5 metric scoring system. The taxa-based PTI index developed for high salinity waters is set on a 0 - 1 scale, with 1 being most like Reference and 0 least like Reference. Taxa abundances that do not qualify for a score of 1 or 0 are not scored (Null). While 77 family- and genus-level taxa having scoring protocols, only a few taxa typically trigger the scoring protocols in a given sample. A PTI index value (average of taxa scores) is calculated if two or more taxa can be scored in a sample. Following the usual practice for this type of index, a score greater than or equal to the mid-point of the index scale (i.e., 0.5) is considered Reference-like.

Strengths:

- High PIBI and PTI scores and high frequencies of desirable phytoplankton attributes occur in Reference water quality conditions. These conditions reflect CBP programmatic goals for water quality.

[5] Spring and summer high salinity (>10‰) waters

Weaknesses:

- Low salinity (<10 ‰) waters in Chesapeake Bay do not have many instances of Reference water quality conditions.

James River Status

PIBI and PTI scores show that phytoplankton community composition is currently unbalanced and undesirable in the tidal James River. Between 1985 and 2012, spring PIBI scores in the James River averaged 2.51, 2.37, and 2.33 in the tidal fresh, oligohaline, and polyhaline assessment segments, respectively. Summer scores averaged 1.34, 1.83, and 2.66, respectively. All the averages rank Poor (<2.0) or Poor-Fair (2.0 – <2.67) on the PIBI scale of 1.0 – 5.0. Variability in the ratings was relatively high. Fair or higher ratings (> 2.67) occurred in 25.2% of individual samples in the tidal fresh segment, 22.0% in the oligohaline segment, and 42.3% in the polyhaline segment. For the taxonomic PTI index, spring scores averaged 0.38 and summer scores averaged 0.41 at the tidal James River polyhaline station LE5.5/LE5.5-W. Both of the seasonal scores indicate undesirable compositions.

V. Chlorophyll *a*

An individual parameter such as the photopigment chlorophyll *a* can be useful in environmental assessments if it is able to indicate the status (health) of biological populations. Chlorophyll *a* is measured with rapid, inexpensive, reliable techniques. It is a recognized proxy for phytoplankton total biomass in open water environments and is used as such to investigate phytoplankton responses to water quality conditions and higher trophic levels (e.g., grazing rates). Researchers rely on chlorophyll *a* concentrations to calculate rates of photosynthesis from C^{14} uptake and dissolved oxygen (light-dark bottle) measurements. Although multi-metric indices such as the PIBI and PTI are better measures of a phytoplankton population's status, the properties of a chlorophyll *a* data set drawn from that population also can indicate status. Specifically, frequent high chlorophyll *a* values conclusively indicate MPL and Degraded water quality and the almost total absence of phytoplankton Reference populations whereas persistent low chlorophyll *a* values usually, but not conclusively, indicate the presence of Reference populations. Multiple analyses of Chesapeake Bay data have established chlorophyll *a* levels representative of phytoplankton reference populations (**Table 2**).

Chlorophyll's potential as an indicator of biotic integrity was recognized by CBP, which suggested narrative criteria (USEPA 2003) and encouraged development of numeric criteria (USEPA 2007b) for Chesapeake Bay and tidal tributaries. In 2005, the Virginia State Water Control Board adopted a narrative chlorophyll criterion for all tidal waters of the state and numeric chlorophyll *a* criteria for the tidal James River (Virginia Water Quality Standards 9 VAC 25-260). The numeric criteria are expressed as seasonal geometric means. Their values do not align with the mean and median concentrations observed in phytoplankton from the various Chesapeake reference, benchmark, and historical water quality conditions (**Table 2**). Instead, they align more closely with the upper percentiles of these populations, suggesting they are not protective of reference populations.

Chlorophyll a Statistics

Statistics often used to analyze Chesapeake chlorophyll *a* are measures of central tendency (e.g., mean, median, geometric mean), variability (e.g., ranges, percentiles, standard deviation) and threshold exceedance (e.g., frequency distributions, conditional probability). Each statistic can be useful in exploring the data. The James River Chlorophyll *a* Criteria Study needed to answer the specific question "are the current criteria, as they appear in regulation, protective?" Data analyses exploring chlorophyll

Table 2. Chlorophyll *a* central tendencies and upper limits in reference water quality conditions or time periods in Chesapeake Bay above-pycnocline waters, by season and salinity zone. Season: spring = March – May; summer = July – September. Salinity zone: TF = tidal fresh (0 - 0.5 ‰); OH = oligohaline (>0.5 – 5 ‰; MH = mesohaline (>5 – 18 ‰); polyhaline (>18 %). Sources: 1 – Olson (2002); 2 - Buchanan *et al.* (2005); 3 – calculated from data used in Buchanan (2014); 4 – USEPA (2007b). Units are µg/liter. Note that the numeric values of the James River chlorophyll *a* criteria, which are currently expressed as geometric means, align more closely with the upper limits.

Central Tendency Season	Salinity Zone	Benchmark "Good" median/mean (1)	Phyto. Reference median (2)	Phyto. Reference median/ mean (3)	Historical (1960s) geometric mean (4)	Range	James R. chlorophyll *a* criteria geometric mean
Spring	TF	3.1/3.5	4.3	3.0/4.3		3.0 – 4.3	10/15
Spring	OH	5.1/5.9	9.7	10.6/12.4	5.8	5.1 – 12.4	15
Spring	MH	6.9/7.2	5.6	5.6/7.8	2.6	2.6 – 7.8	12
Spring	PH	3.4/4.1	2.8	3.6/4.1	1.4	1.4 – 5.0	12
Summer	TF	7.3/6.9	8.6	6.3/8.6		6.3 – 8.6	15/23
Summer	OH	7.8/7.7	6.0	5.8/8.5	14.8	5.8 – 14.8	22
Summer	MH	8.4/7.9	7.3	7.6/8.1	7.3	7.3 – 8.4	10
Summer	PH	4.3/3.7	4.5	5.2/5.3	1.7	1.7 – 5.3	10

Upper Limits (Thresholds) Season	Salinity Zone	Benchmark "Good" 90th%ile (1)	Phyto. Reference 95th%ile (2)	Phyto. Reference 90th/95th%ile (3)	Historical (1960s) 1.2815 SD log-normal (4)	Range
Spring	TF	4.2	13.5	10.4/13.5		4.2 – 13.5
Spring	OH	9.8	24.6	22.6/28.7	18.2	9.8 – 28.7
Spring	MH	11.0	23.8	14.5/21.5	8.0	8.0 – 23.8
Spring	PH	12.9	6.4	6.8/7.3	4.3	4.3 – 12.9
Summer	TF	8.7	15.9	16.9/24.2		8.7 – 24.2
Summer	OH	10.8	24.4	17.2/23.2	45.7	10.8 – 45.7
Summer	MH	11.1	13.5	11.8/13.8	22.6	11.1 – 22.6
Summer	PH	6.0	9.2	7.4/8.0	5.1	5.1 – 9.2

a associations in the context of the current criteria (geometric means) were most helpful to VADEQ. The focus in this section is chlorophyll's ability to indicate biotic integrity of phytoplankton populations.

1. Central tendency

The central tendency of a population of chlorophyll *a* values changes as underlying water quality conditions change. Arithmetic and geometric means of chlorophyll *a* are below 11.5 µg/liter combined REF+MBL water quality conditions, and between 3.9 and 41.4 µg/liter in MPL, Degraded, and Very Degraded conditions (**Table 3**). The two groups overlap between 3.9 - 11.5 µg/liter; high means conclusively indicate the presence of degraded water quality conditions. Similar results are found for phytoplankton chlorophyll *a* in other configurations of reference conditions (e.g., those listed in **Table 2**).

Strengths:
- Means and medians are the most accurate statistics when sample sizes are small.
- Station-specific arithmetic and geometric means of a chlorophyll *a* data set are closely related regardless of water quality conditions (Appendix A). Linear relationships between the two can be used to convert observed arithmetic means to corresponding geometric means, and vice versa.

Weaknesses:
- Means and medians do not *directly* indicate the magnitude or frequency of the highest chlorophyll *a* values ("algal blooms"), which are the values that concern VADEQ (but see below).
- The arithmetic mean assumes data are randomly (normally) distributed; the geometric mean assumes data are log-normally distributed. When these underlying assumptions are not met in the data, these two measures of central tendency can misinform. The median does not presume any specific distribution.

2. Variability and threshold exceedance rates

Individual measurements of low chlorophyll *a* concentrations are observed across all water quality categories while high concentrations (algal blooms) are only observed in the MPL and Degraded categories. Individual measurements of low concentrations usually, but not conclusively, indicate the presence of phytoplankton Reference populations. The occurrence of many high chlorophyll measurements conclusively indicate the absence of phytoplankton Reference populations.

The combined REF+MBL categories can be considered as meeting the programmatic water quality goals for Chesapeake Bay open waters, even though concentrations of one or both nutrients in MBL exceed the bloom-limitation thresholds to some extent. The upper limits (90th percentiles) of chlorophyll *a* concentrations in these desirable water quality categories are between 7.4 and 19.8 µg/liter, depending on season and salinity zone (**Table 4**). In MPL and Degraded conditions, upper limits range higher than 70 µg/liter and individual chlorophyll *a* measurements exceed the REF+MBL upper limits as often as 42% of the time. In Very Degraded conditions, the upper limits and exceedance rates can be low because phytoplankton growth is inhibited by poor light to a point where chlorophyll *a* levels resemble those in REF+MBL (see more below).

As mentioned above, the numeric values of the James River chlorophyll *a* criteria align closely with the upper percentiles rather than the central tendencies of chlorophyll *a* concentrations observed in various reference, benchmark, and historical water quality conditions (**Table 2**). If the criteria were expressed as upper limits instead of geometric means, the current numeric values would be generally protective of reference phytoplankton populations. Individual chlorophyll *a* measurements in the combined REF+MBL conditions would only exceed the criteria 2% - 22% of the time, and when they did it would be only by 2X – 3X. However, because the numeric values of the criteria are expressed as geometric means, they allow individual measurements to exceed the upper limits of reference populations 25% to more than 70% of the time, depending on season and salinity.

Strengths:
- Measures of chlorophyll *a* variability and threshold (upper limit) exceedance rates are better indicators of magnitude and algal bloom frequency than measures of central tendency.

Weaknesses:
- Threshold (upper limit) exceedance rates are accurate only when sample size is comparatively large.

Table 3. Arithmetic means (Avg) and geometric means (Geomean) of chlorophyll *a* from open water environments, grouped by season, salinity zone, and water quality category. Chlorophyll *a* values are typically log-normal distributed, so the geometric mean (highlighted) more accurately reflects the central tendency of a "population" of values. The arithmetic mean is included here for comparison. Water quality categories: Reference (REF), adequate water clarity and bloom-limiting concentrations of DIN and PO_4; Mixed Better Light (MBL), adequate water clarity and excess concentrations of one or both nutrients; Mixed Poor Light (MPL), inadequate water clarity and bloom-limiting concentrations of one or both nutrients (many samples appear to support peak bloom or post-bloom phytoplankton communities); Degraded (DEG), inadequate water clarity and excess concentrations of both nutrients; Very Degraded = extreme subset of Degraded. Salinity zone: TF = tidal fresh (0 - 0.5 ‰); OH = oligohaline (>0.5 – 5 ‰; LoMH = low mesohaline (>5 – 10 ‰); HiMH = high mesohaline (>10 – 18 ‰); polyhaline (>18 %). *, fewer than 10 data points were found in this category in 1984-2013 Chesapeake Bay tidal waters. Units: µg/liter.

	Reference (REF)		Mixed Better Light (MBL)		Mixed Poor Light (MPL)		Degraded (DEG)		Very Degraded (Subset of DEG)	
Salinity Zone	Avg	Geo-mean	Avg	Geo-mean	Avg	Geo-mean	Avg	Geo-mean	Avg	Geo-mean
Spring (March – May)										
TF	*	*	4.8	3.4	23.2	17.3	11.5	7.1	7.8	5.4
OH	*	*	11.4	9.5	24.3	18.8	10.8	7.4	6.6	4.7
LoMH	*	*	9.7	8.1	20.7	16.2	18.8	11.1	11.8	7.8
HiMH	7.8	6.5	8.3	6.6	16.8	13.2	11.9	8.5	10.4	6.5
PH	4.1	3.6	4.9	4.0	8.5	7.0	4.4	3.9	7.8	6.3
Summer (July – September)										
TF	*	*	8.6	6.4	41.4	33.6	24.1	16.2	15.6	9.8
OH	10.5	7.8	6.8	5.6	34.1	27.3	10.9	8.0	15.8	11.4
LoMH	10.8	10.4	10.5	9.3	21.4	17.6	11.7	9.7	15.6	11.6
HiMH	7.6	7.2	6.8	6.2	12.4	10.8	8.6	7.3	11.2	9.1
PH	5.3	5.0	4.7	4.3	8.8	7.9	6.1	5.5	8.6	6.9

Table 4. The upper limits, or 90^{th} percentiles, of chlorophyll *a* in the combined REF+MBL water quality categories for the given season and salinity zone rounded to the nearest whole number. These water quality categories are considered as meeting narrative programmatic goals for nutrients and water clarity. Data are from the above-pycnocline layer in open water habitats of Chesapeake Bay and its tidal tributaries, 1984-2013. Sample sizes in parentheses indicate these least-degraded conditions are not uncommon in Chesapeake tidal waters and are thus attainable. Units: µg/liter.

	Tidal Fresh	Oligohaline	Low Mesohaline	High Mesohaline	Polyhaline
Spring	11 (n=470)	20 (n=175)	18 (n=452)	16 (n=1,020)	8 (n=335)
Summer	17 (n=524)	13 (n=187)	16 (n=184)	11 (n=958)	7 (n=701)

The standard deviation assumes data are randomly (normally) distributed; the geometric standard deviation assumes data are log-normally distributed. When these underlying assumptions are not met in the data, these measures of statistical dispersion, or variability, can misinform. (Interquartile ranges and percentiles do not presume a specific distribution.)

Relationships Between Chlorophyll a Statistics

In large chlorophyll data sets, strong relationships occur between the central tendency and upper limits, and between the central tendency and exceedance rates of a threshold (Appendix B). These relationships have been found in low-frequency fixed station CBP data, in high-frequency spatially-rich DATAFLOW data, and in high-frequency temporally-rich continuous monitoring (ConMon) data (**Figure B-1**). As long as sample sizes are large, the relationships are strong regardless of how the data are grouped for analysis (e.g., by water quality category, by station, by salinity regime), or what measure of central tendency is used, or what threshold or measure of upper limits is used (e.g., **Figure B-2 – B-11**).

Empirical relationships such as these allow analysts to estimate the mean concentration associated with an allowable exceedance rate of a specific chlorophyll *a* threshold. For example, if a chlorophyll *a* threshold (upper limit) of 23 µg/liter is applied and a 10% exceedance rate is allowed, the highest allowable arithmetic mean in the associated 'population' of low-frequency, fixed-station chlorophyll *a* data is ~12.7 µg/liter, the highest allowable geometric mean is ~9.6 µg/liter, and the highest allowable median is ~9.6 µg/liter (extrapolated from the bottom graphs in Appendix B **Figures B-2 – B-5**).

The upper limits (e.g., 90th percentiles) of the distribution of chlorophyll *a* concentrations expected for each of the James River criteria can be calculated from the relationships in Appendix B **Figures B-9** and **B-11.** Results are shown in **Table 5** (and **Table ES-1**). Waters that just meet the James River criteria can be expected over the long-term to exceed these upper limits roughly 10% of the time.

Over long periods at a station or in water quality categories, or when many data points are collected with high-frequency sampling methods (e.g. Robertson 2015), chlorophyll *a* measurements tend to exhibit log-normal distributions. So in most cases, the median and geometric mean are more appropriate to use as measures of central tendency than the arithmetic mean. All three statistics were examined here and were shown to have similar, close relationships with the frequency of exceeding upper limits, or thresholds.

Table 5. The current James River chlorophyll *a* criteria (geometric means) and the projected upper limits (90th percentiles) of chlorophyll *a* concentrations associated with attainment of the criteria, in µg/liter.

Salinity zone	Numeric values of James River chlorophyll *a* criteria	Upper limits with attainment of James River criteria
	Spring (March – May)	
TF	10 (upper)	27
	15 (lower)	40
OH	15	40
MH	12	33
PH	12	33
	Summer (July – September)	
TF	15 (upper)	29
	23 (lower)	45
OH	22	43
MH	10	20
PH	10	20

Conditional Probability

Conditional probability is the probability that an event will occur under specific conditions. It can be used to determine the frequency of exceeding some threshold of concern in a given condition, for example the frequency of exceeding a chlorophyll *a* threshold when chlorophyll *a* concentrations are < 5 µg/liter. The strong relationships between chlorophyll *a* central tendency, upper limits, and threshold exceedance frequencies lend themselves to this kind of analysis because they hold across all water quality categories (e.g., upper panels, **Figure B-2 – B-4**). Conditional probability may also work well for calculating threshold exceedances for chemical parameters such as pH and dissolved oxygen, for example the frequency of exceeding a pH of 9 when for a given chlorophyll *a* increment. However, phytoplankton taxonomic composition and many community characteristics change as water quality conditions change, and low chlorophyll *a* concentrations do not necessarily indicate good phytoplankton communities.

Specifically, phytoplankton populations with low chlorophyll *a* values in Degraded water quality conditions are associated with undesirable phytoplankton features that contrast with low-chlorophyll populations in Reference conditions (see section IV above). Low-chlorophyll populations in Degraded conditions have lower taxa richness, relatively high proportions of nuisance and/or toxin-producing taxa, and a prevelence of taxa with inate abilities to regulate their vertical distributions (dinoflagellates, blue-green algae). They are typically associated with higher pheophytin and DOC concentrations, indicating cellular physiological stress. Chla:C ratios (chlorophyll *a* cell content) are also higher, indicating poor water clarity is inhibiting phytoplankton photosynthesis and growth over time.

At higher chlorophyll *a* concentrations, potentially harmful taxa occur more frequently in Degraded samples than in Reference samples. This seems to be a function of higher cell abundances as well as the effects of Degraded water quality. As chlorophyll *a* increases, so does the number individual taxa in the phytoplankton sample that exhibit comparatively high abundances, or "taxa blooms" (**Figure 2**). This occurs regardless of water quality condition. However, as indicated by PTI index scores, undesirable taxa occur more often and in higher maximal abundances in Degraded conditions (Buchanan 2015a). Consequently, a larger proportion of the taxa blooms in Degraded conditions will be comprised of undesirable taxa. In higher salinity waters, these include the toxin-producers *Anabaena* sp (blue-green), *Prorocentrum minimum* and *Cochlodinium* spp. (dinoflagellates), and *Pseudo-nitzschia seriata* (diatom).

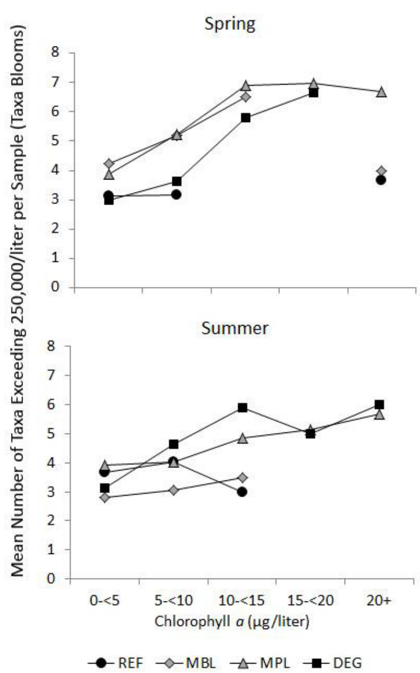

Figure 2. Mean number of taxa blooms *versus* chlorophyll *a* concentration in high salinity waters. This is Figure 7 in Buchanan (2015a).

As a result of population differences in Reference and Degraded conditions, many phytoplankton community metrics and the two indices can differ substantially in a given chlorophyll *a* increment. For example, the probability of PIBI scores $\geq$ 2.67 (Reference-like populations) is much higher in the Reference water quality category for chlorophyll increments experienced in both Reference and Degraded conditions. While useful and informative, conditional probability results for many phytoplankton metrics across a range of chlorophyll *a* increments should be used with caution to ensure that underlying factors such as water quality conditions are not affecting the probabilities. This is especially true in the James River environment, which has eutrophic water quality. Phytoplankton communities are expected to change significantly with nutrient and sediment reductions. More on the issue in Appendix C.

VI. Deleterious Algal Blooms

The CBP (USEPA 2003, 2007b) lists the potential deleterious effects of algal blooms in Chesapeake Bay:

- Excess dead algae are consumed by bacteria, which remove oxygen from the water column in the process and create hypoxic and anoxic layers.
- Blooms can be dominated by a single species, which can represent poor food quality or can produce toxins that impair the grazers feeding directly on them.
- Large blooms can reduce light penetration, or water column clarity.

These findings come from literature reviews, Chesapeake Bay water quality model output, and analyses of the very large water quality database of monitoring data collected in the Bay since the 1960s. They corroborate the documented impairments of estuarine aquatic life, recreation (swimming, boating), and the production of edible, marketable natural resources (fish, shellfish) by large algal blooms found elsewhere. The objective of the James River Chlorophyll Criteria Study is to further investigate potential deleterious impacts of algal blooms on James River water quality and biological communities. An 'effects-based' approach was used in that study, with the intention of identifying chlorophyll thresholds above which deleterious effects occur. Although the study is not complete as of this writing, one product—an analysis of the study's James River monitoring data—is showing that elevated chlorophyll *a* concentrations are indeed associated with a range of deleterious effects (Bukaveckas, in prep.).

James River Chlorophyll a Criteria Study

A combined probability approach was used in the Study to derive protective chlorophyll *a* thresholds for a variety of water quality and biological parameters. The observed frequency of a water quality or biological metric exceeding a given threshold in a chlorophyll *a* increment is weighted by the frequency of that chlorophyll *a* increment occurring in a given season-year of the study. In other words, the threshold exceedance frequency in each chlorophyll *a* increment (conditional probability) is multiplied by the frequency of the condition (chlorophyll *a* increment). The weighted probabilities for all chlorophyll increments in a season-year are summed and the combined probability is paired with the arithmetic mean chlorophyll *a* concentration for the same period. The approach shown in **Figure 4** is then used to identify different levels of risk and propose 'defensible' ranges. Means falling above line B are associate with a relatively high risk of harm from algal blooms. Means falling in the range between A and B are considered defensible. The protectiveness of a criterion falling in the defensible range is to some extent uncertain but the relative risk of harm from algal blooms is lower. Means falling at or below line A are judged protective against the harmful effects of algal blooms. Defensible ranges were determined for eight of the ten season-segment combinations in the tidal James River.

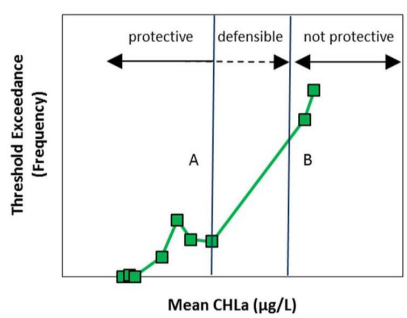

Figure 4. The combined probability of threshold exceedance (e.g., pH > 9) vs the seasonal arithmetic means of chlorophyll *a* for the study period are used to identify ranges of chlorophyll *a* that are protective, 'defensible' or not protective. Figure 4 in Bukaveckas (in prep.).

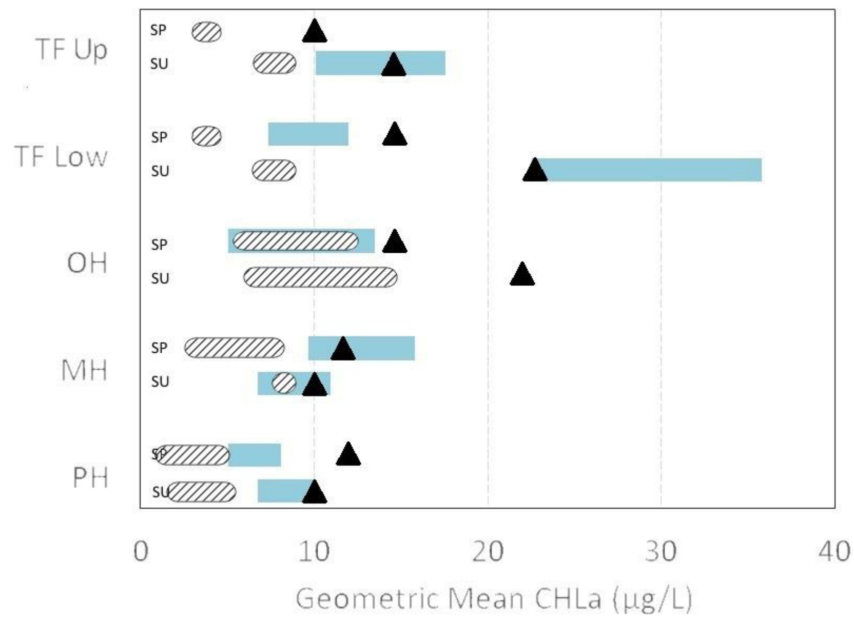

Figure 5. James River Chla criteria (▲), the range of 'defensible' geometric means (blue) derived from Bukaveckas (in prep.), and the full range of means and medians (hatched) identified as protective of various reference populations (from **Table 2** above) for the five tidal James River segments, spring (Sp) and summer (Su). The 'defensible' ranges were expressed as arithmetic means in Bukaveckas (in prep.) and converted to geometric means for this figure using the relationships shown in **Appendix A.**

The James River chlorophyll *a* criteria are expressed as geometric means while the Study's defensible ranges are expressed as arithmetic means. To compare the defensible ranges to the James River criteria, the ranges need to be translated from arithmetic means to geometric means. This was not done in Bukaveckas (in prep.), but it can be done using the graphs in Appendix A. The projected geometric means that delineate the ranges are then compared to the James River chlorophyll *a* criteria. Results are shown in **Figure 5**.

Protective Thesholds

Four James River criteria fall at or above their defensible ranges (spring TF low, spring OH, and spring and summer PH) and thus are not protective against any of the algal impacts represented by the defensible ranges. Three fall inside their defensible ranges (summer TF up, spring and summer MH) and could be considered protective against some algal bloom impacts. Closer examination of study results for these three season-segments suggests the James River criteria may be somewhat protective against elevated microcystin (TF) and elevated pH and low dissolved oxygen (MH). Only one of the James River criteria (summer TF low) is at the bottom of its defensible range and technically could be considered protective against algal bloom impacts. However, that criteria and its defensible range seem unusually high compared to the other criteria and ranges. Defensible ranges for two season-salinities (spring TF upper, summer OH) could not be determined.

Similarly mixed results are found when James River criteria are compared to effects-based chlorophyll *a* means and upper limits derived from Chesapeake-wide analyses and presented as protective against algal bloom impacts in USEPA (2003 and 2007b) and Harding *et al.* (2014). For example, the criteria for summer mesohaline and polyhaline overlap the May – August geometric means considered protective against dissolved oxygen impairment in deep waters of the lower Chesapeake mainstem (7 - 11 µg/liter). In low salinities, the summer criteria are at or above the geometric means protective against local *Microcystis* impacts (15 µg/liter). They are distinctly higher than the geometric means protective against water clarity impairment in low salinity waters (12 µg/liter); they are closer but still higher than the geometric means protective in high salinity waters (8 µg/liter). In spring, the low salinity criteria are not protective against *Prorocentrum minimum* greater than 3000 cells per milliliter, or levels that potentially harm shellfish.

The defensible ranges of the James River Study can also be compared to the ranges of chlorophyll *a* means and medians characteristic of phytoplankton reference populations, shown has hatched ovals in **Figure 5**. Six of the eight defensible ranges are higher than their corresponding reference ranges while two overlap. All of the James River criteria fall above the reference population ranges. Clearly none of the James River criteria, and possibly only two of the 'defensible' ranges, are protective *cf* the various phytoplankton reference populations for Ches.

The Study's assessments of protectiveness against algal bloom impacts are based on empirical relationships found in the recent monitoring data, and Bukaveckas (in prep.) recognizes these relationships between chlorophyll *a* and the various response metrics are subject to change as TMDL-mandated nutrient and sediment load reductions take effect. Indications of the anticipated changes can already be seen in comparisons of various phytoplankton indices and metrics in Reference and Degraded conditions (Appendix C). In Degraded water quality conditions, effects-based thresholds for chlorophyll *a* are at times hard to detect and tend to appear at relatively high concentrations. Chlorophyll *a* concentrations in Reference water quality conditions are typically below these effects-based thresholds. However, different—lower—thresholds sometimes appear in Reference conditions. For example, PIBI thresholds are difficult to find in Degraded conditions but seem to appear at 5 – 10 µg/liter in spring and 10 – 15 µg/liter in summer in REF+MBL conditions (**Figure C-2**).

VII. Chlorophyll *a* Criteria

Water quality standards are supposed to impart protection of a resource so that waters of the United States support aquatic life and are fishable and swimmable. Virginia adopted a narrative chlorophyll *a* criterion for all state tidal waters and numeric criteria for the tidal James River in 2005. In justifying the need for numeric criteria for the James River, VADEQ recognized that "chlorophyll *a c*riteria are derived to protect for balanced aquatic plant life populations and against the overgrowth of nuisance, potentially harmful algal species" (VADEQ 2004, pg. 5). In other words, protection *cf* aquatic life and protection *against* algal bloom impacts. In the EPA 2007 chlorophyll addendum for Chesapeake Bay water quality criteria, EPA encourages states to adopt at a minimum numeric criteria based on harmful algal bloom impacts. States can also adopt numeric criteria based on concentrations in reference conditions.

Protective numeric chlorophyll *a* thresholds considered by CBP (USEPA 2003, 2007b) and VADEQ (2004) come from multiple lines of evidence, some involving analyses of historic and current reference conditions. Chesapeake-based results in USEPA (2003, 2007b), Olson (2002), Buchanan *et al.* (2005), Marshall *et al.* (2006), Harding *et al.* (2014), Bukaveckas (in prep.), and elsewhere are revealing a continuum of protective chlorophyll *a* thresholds, with protection of phytoplankton reference populations achieved at the lower end and protection against various algal bloom impacts achieved at the higher end.

Phytoplankton reference populations are presently the best available representation of balanced, indigenous, desirable phytoplankton aquatic life. Their chlorophyll *a* concentrations (**Table 2**) are considered to be fully achieving the narrative criterion, which is:

> "Concentrations of chlorophyll *a* in free-floating microscopic aquatic plants (algae) shall not exceed levels that result in ecologically undesirable consequences—such as reduced water clarity, low dissolved oxygen, food supply imbalances, proliferation of species deemed potentially harmful to aquatic life or humans or aesthetically objectionable conditions—or otherwise render tidal waters unsuitable for designated uses (Virginia Water Quality Standards 9 VAC 25-260)."

Chlorophyll *a* thresholds protective against algal blooms are higher. Achieving these thresholds should help Chesapeake tidal waters avoid the immediate impacts of algal blooms (low DO, high pH, toxicity) and partially restore many of the ecological functions of phytoplankton populations. These thresholds, however, might not adequately protect a fully functioning phytoplankton population that can absorb and retain nutrients efficiently and provide a stable, sustainable food source for higher trophic levels.

The current chlorophyll *a* criteria for the tidal James River, expressed as geometric means, are positioned at or above the high end of the continuum of protective thresholds. They are not protective of phytoplankton reference populations, and in many instances they are not protective against algal bloom impacts. Thus, they do not achieve the narrative chlorophyll *a* criteria recommended in USEPA (2003) and adopted into Virginia's water quality standards.

The Water Clarity Connection

Chlorophyll *a* criteria alone will not ensure protection of desirable phytoplankton populations. As stated earlier, high chlorophyll *a* levels by themselves conclusively indicate the absence of Reference communities but persistent low chlorophyll *a* levels do not conclusively indicate the presence of Reference communities. James River chlorophyll *a* criteria can sometimes be attained in very Degraded conditions. In these conditions, poor water clarity is negatively impacting phytoplankton community function and structure to a point where growth is light-limited except at the very surface. Total biomass, expressed as chlorophyll *a*, remains low even in nutrient-enriched waters and the dominant taxa tend to be those best adapted for low light conditions, e.g., dinoflagellates and blue-greens.

Water clarity in Chesapeake Bay is controlled largely by factors other than phytoplankton.[6] As water clarity improves, phytoplankton are eventually released from light limitation and nutrients assert more control on their growth. Algal blooms form rapidly if the release from light limitation occurs in nutrient-enriched conditions; phytoplankton growth is slower and more controlled if the release from light limitation occurs in nutrient concentrations that approach bloom-limiting levels. When water clarity meets phytoplankton light requirements for unstressed photosynthesis, chlorophyll *a* concentrations that consistently fall below the upper limits shown in **Table 4** reliably indicate the presence of Reference and MBL populations.

Threshold criteria for both water clarity and chlorophyll *a* in Chesapeake tidal waters could better protect phytoplankton Reference communities and by extension balanced, indigenous, desirable aquatic

[6] Empirical models of light attenuation in Chesapeake Bay developed by Xu *et al.* (2005), USEPA (2008), Robertson (in prep.), and others demonstrate the usually larger influences of colored dissolved organic matter, non-algal suspended solids, and water itself.

life in open water environments. This is not unexpected, given that multiple environmental factors usually control biological populations. Habitat requirements for healthy SAV are characterized by thresholds for TSS, DIN, DIP, and phytoplankton chlorophyll *a* as well as water clarity (Batiuk *et al.* 1992, 2000). Habitat requirements for healthy benthic macroinvertebrates are characterized by few pollution sources in adjacent watersheds and thresholds for sediment organic content and toxic contaminants as well as dissolved oxygen (Weisburg *et al.* 1997). The use of multiple measures and criteria to assess ecosystem health is encouraged (e.g., Schulze 1999, Simon 2003, Martinez-Crego *et al.* 2010). USEPA (2003) states:

> "The three Chesapeake Bay criteria–dissolved oxygen, water clarity and chlorophyll *a*–should be viewed as *an integrated set of criteria* applied to their respective sets of designated use habitats and addressing similar and varied ecological conditions and water quality impairments. They provide the basis for defining the water quality conditions necessary to protect the five essential Chesapeake Bay tidal-water designated uses."

All three sets of Chesapeake Bay criteria must be met in order for a tidal segment to be delisted.

If criteria—or at a minimum screening thresholds—for water clarity were applied to open water environments, they would address those cases where phytoplankton growth and chlorophyll *a* concentrations are suppressed by extremely degraded underwater light. Expansion to open waters of the SAV-based, shallow water criteria for water clarity (**Table 1**) would partially protect against algal blooms in low salinities and be somewhat protective against algal blooms in high salinities. As long as a segment's open water designated use was impaired for both chlorophyll *a* and water clarity, it would remain on the impaired waters list. Open waters that meet the water clarity criteria but not the chlorophyll *a* criteria could potentially be "partially delisted." Open waters that meet the chlorophyll *a* criteria but not the water clarity criteria are probably growth-suppressed and should stay listed.

VIII. Choice of a Chlorophyll *a* Criteria Statistic

Most of the numeric values for chlorophyll *a* criteria suggested by EPA (2003, 2007b) are intended as upper limits for individual measurements. In fact, EPA specifically encourages threshold criteria for chlorophyll *a* (USEPA 2007b):

> "A criterion threshold is a concentration that should rarely be exceeded by a 'population' of concentration data exhibiting healthy levels. The state-adopted concentration-based chlorophyll *a* criteria values are threshold concentrations that should only be exceeded infrequently (e.g., <10%) ..." (p. 71).

High chlorophyll *a* concentrations are of concern to VADEQ because they are associated with nuisance and harmful algal blooms. They are also good indicators of Degraded conditions (see above). Threshold criteria based on the upper limits of desirable chlorophyll *a* levels can detect frequent occurrences of individual high values that are potentially harmful. High-resolution data such as the temporally-rich ConMon data and spatially-rich DATAFLOW data are particularly suited for upper limit thresholds, and analysis methods currently being developed will make better use of these data (e.g., Robertson in prep.). While it is not clear if routine monitoring with DATAFLOW and ConMon will continue in Virginia, satellite imagery is evolving as yet another high resolution data source.

Virginia uses the geometric mean instead of an upper limit threshold as its chlorophyll *a* statistic. This choice stems in part from VADEQ's past reliance on low-resolution, fixed station monitoring data. These data are collected during routine monitoring cruises from 1 m below the surface or from multiple depths in the above-pycnocline layer (depth integrated). A recent baywide compilation of fixed station monitoring data from open waters (station depth > 2 meters), collected in spring and summer between 1984 and 2013, found 64,200 samples (Buchanan 2014). While this total is impressive, the number of measurements in an assessment unit (segment) in Virginia's 3-year assessment window is too few to accurately estimate the frequency of exceeding an upper threshold. So, for fixed station data, a central tendency statistic such as the geometric mean has proved most useful. Modeling nutrients and chlorophyll is also easier with seasonal means than with individual measurements (Robertson, pers. comm.).

If the data available from routine assessments helps to determine which criteria statistic is most appropriate to apply, then the resulting choice of statistic will help decide what numeric values for the criteria are adequately protective of the desired outcome(s). If high-resolution chlorophyll *a* data (e.g. DATAFLOW, ConMon, satellite imagery) are routinely available, a criterion based on upper limits of allowable chlorophyll *a* concentrations would increase the accuracy and sensitivity of assessments. If high-resolution data are not available, statistical relationships between chlorophyll *a* central tendency and upper limits associated with allowable exceedance rates could be used to identify seasonal means that correspond to acceptable risk. For example, if an upper limit threshold of 30 µg/liter is not to be exceeded more than 10% of the time in spring, the geometric mean that would match this exceedance rate is a geometric mean of 11 µg/liter (from Appendix B **Figure B-9**). Either or both statistics could be used as criteria.

If Virginia continues to rely on its fixed station monitoring program and use a seasonal geometric mean as its chlorophyll *a* criteria statistic, the numeric values of the current criteria should be reduced to values that are, at a minimum, more protective against algal bloom impacts. Ideally, the values would be further lowered to levels protective of phytoplankton chlorophyll *a* in REF+MBL conditions, and by extension balanced, indigenous, desirable aquatic life. Changing the numeric values of the criteria will require changing the established water quality standard. VADEQ will then want to reevaluate its current assessment methodology (see below).

If Virginia changes its criteria statistic from a geometric mean to an upper limit, the numeric values of the current criteria would be in large part protective of reference populations and protective against algal bloom impacts. This is because the numeric values of the criteria are similar to the 90^{th} - 95^{th} percentiles of chlorophyll *a* concentrations in the combined REF+MBL categories (**Table 2**). Making this change in the criteria statistic will require changing the established water quality standard and will likely require changes in the current assessment methodology. The criteria would be most appropriately applied to high-resolution data sets.

Choice of Assessment Reference Curve

The choice of a criterion's statistic and its numeric value should in theory be decided apart from the assessment method. However, the level of protectiveness of the chosen criteria will depend on assessment methodology, and particularly on the assessment reference that is applied. Choice of the criteria statistic should match the assessment reference in order for assessments to work as intended.

EPA (2007a) recommends applying the Cumulative Frequency Distribution (CFD) procedure to determine criteria exceedances. In lieu of biology-based assessment reference curves, EPA (2007a) recommends using a 10% hyperbolic curve as the default curve with the CFD method. The default curve shows allowable criteria exceedances (10%) over space (x-axis) and time (y-axis). It provides equal weight to exceedances occurring in either time or space, and is consistent with past EPA national guidance on allowable exceedances.

The 10% hyperbolic curve is a suitable reference assessment curve when 90% or more of a population of data are expected to fall below the criterion at attainment and only 10% are allowed to exceed it at attainment. When upper limits (90^{th} and 95^{th} percentiles) of phytoplankton reference populations are used as exceedance thresholds, the resulting biology-based CFD assessment reference curves closely track the 10% hyperbolic curve (Buchanan 2014; **Figure D-4** in Appendix D). The James River chlorophyll *a* criteria, however, are expressed as geometric means and not upper limits. By nature of the fact that the geometric mean is a measure of central tendency calculated from log-transformed values, almost half the values comprising the mean can be expected on average to exceed the criterion when the geometric mean reaches attainment. Therefore, a CFD assessment reference curve measuring attainment of a geometric mean criterion differs from the 10% hyperbolic curve and allows almost half of the data points to exceed the criterion at attainment.

When James River chlorophyll *a* is assessed with the current VADEQ methodology (i.e., criteria expressed as geometric means, CFD assessment method, and default 10% hyperbolic curve), the default curve forces at least 90% of the measured *geometric means* to fall below the criterion before attainment can be declared. The stringency imposed by the default curve is balanced to some extent by the higher numeric values of the James River chlorophyll *a* criteria, and the net effect is protection of phytoplankton reference populations to varying degrees (Appendix D). This inadvertent consequence of method choices is beneficial because it makes the existing assessment methodology somewhat protective of reference populations, but it seems unintended. The choices of criteria statistic, assessment methodology, and assessment reference curve should complement each other.

Conclusions

Narrative programmatic goals for Chesapeake Bay water quality can be directly linked to science-based, numeric values for phytoplankton chlorophyll *a* criteria. A logical progression of steps building on principal ecological concepts and substantiated with empirical evidence collected throughout the Chesapeake Bay demonstrates this connection. Open water environments that meet phytoplankton habitat requirements for nutrients and water clarity will attain their designated uses and sustain ecologically desirable reference phytoplankton populations. These populations are achievable representations of the balanced, indigenous aquatic life called for in Virginia Water Quality Standards. Chlorophyll *a* concentrations in these populations attain CBP and VADEQ narrative criteria for chlorophyll *a*.

The numeric values of Virginia's current chlorophyll *a* criteria are not protective of phytoplankton reference populations. They are not protective of baywide water clarity requirements for healthy underwater grasses in near-shore waters. The criteria may not be protective against potential harm from toxin producing algal taxa, including *Microcystis* and *Prorocentrum*. The criteria are not protective of four of the eight season- and segment-based "defensible ranges" proposed in the ongoing James River Chlorophyll *a* Criteria Study, but may be somewhat protective of four other defensible ranges. The criteria may be somewhat protective of the dissolved oxygen requirements for healthy benthic macroinvertebrate populations in deep waters of the Bay mainstem. Making the current assessment methodology more stringent might increase protectiveness but it is not likely to make the criteria fully protective of all designated uses.

Virginia's current chlorophyll *a* criteria for the James River (10 - 23 μg/liter, depending on season and segment) are expressed as seasonal geometric means over 3-year assessment periods. If Virginia decides to continue to rely on the geometric mean statistic for its chlorophyll *a* criteria, one option for making

Table 6. Chlorophyll *a* geometric means (ug/liter) of high-quality phytoplankton populations observed in REF + MBL water quality categories.

Season / Salinity Zone	Geometric Mean
Spring	
Tidal fresh	3.4
Oligohaline	9.5
Low Mesohaline	8.1
High Mesohaline	6.6
Polyhaline	3.8
Summer	
Tidal fresh	6.4
Oligohaline	5.7
Low Mesohaline	9.5
High Mesohaline	6.7
Polyhaline	4.3

the criteria more protective is to lower the numeric values of the criteria. Geometric means of phytoplankton populations observed in Reference (REF) and the near-Reference Mixed Better Light (MBL) water quality categories are shown in **Table 6.** Criteria closer to these values would make Virginia standards more protective ecologically desirable phytoplankton populations. Another option would be to change the criteria statistic from a geometric mean to an exceedance threshold while leaving the numeric values of the current criteria approximately the same. The current numeric values are more appropriately viewed as upper limits or thresholds for individual measurements of chlorophyll *a* concentration. When applied as exceedance thresholds instead of geometric means, these values are generally protective of ecologically desirable phytoplankton populations. Both options would involve changing Virginia's established water quality standards. They should include a reevaluation of Virginia's criteria assessment methodology and assessment references. Multiple methodologies will probably be needed if high-frequency data types (DATAFLOW, ConMon, satellite imagery) are included in future assessments.

The current VADEQ assessment methodology calculates the cumulative frequency distribution of the percent of seasonal geometric means that fail the criteria, and compares the results to the default assessment reference which is a 10% hyperbolic curve. Use of the 10% curve as the default forces 90% or more of seasonal *geometric means* to fall below the criteria's numeric values before attainment is declared in a segment over an assessment period. The methodology effectively and significantly increases the protectiveness of the current James River criteria beyond what would be expected by simply examining the criteria's numeric values. It highlights the importance of considering assessment methodology and reference endpoints when selecting numeric criteria.

Chlorophyll *a* criteria alone will not protect aquatic life designated uses in open waters. Very poor water clarity caused by suspended sediments and other non-living matter impedes phytoplankton photosynthesis and growth. If water clarity screening thresholds or criteria are applied to Chesapeake Bay's open water environments in addition to chlorophyll *a* criteria, phytoplankton populations and by extension balanced, indigenous, desirable aquatic life will be fully protected.

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Appendix A

Relationships Between Arithmetic and Geometric Means

Station-specific arithmetic and geometric means for chlorophyll *a* (µg/liter) are very closely related (**Figure A-1**) and their relationship can be used to convert arithmetic means to geometric means, and *vice versa*. Graphs on the right show results for above-pycnocline, 1984 – 2013 data collected at tidal monitoring stations in Chesapeake open water environments (station depth is greater than 2 m). Data are grouped by station and season. Stations with less than 50 data points per season are excluded. The assigned salinity of each station's CBP segment is indicated: TF, tidal fresh; OH, oligohaline; MH, mesohaline; PH, polyhaline. Blue line is the 1:1 line. Black line is the linear regression between arithmetic and geometric mean.

The influence of salinity regime on the relationship seems minimal. Variability in the relationship (i.e., uncertainty) appears to be greatest when values of the means are high.

The James River monitoring stations (circled red in the graphs) do not stand out as different from other Chesapeake Bay stations.

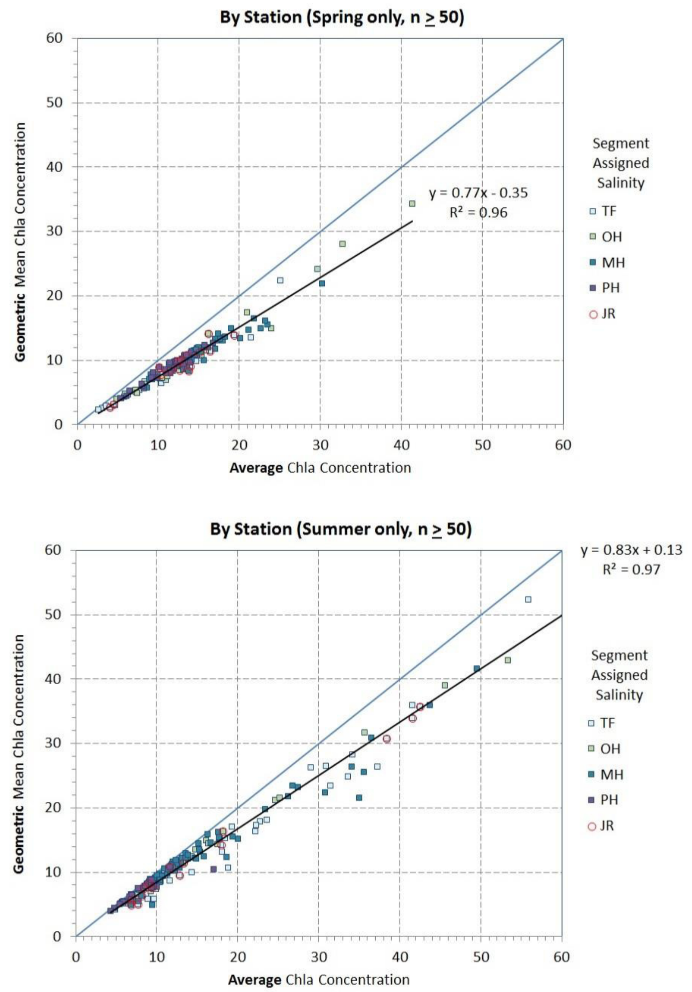

Figure A-1. Relationships between arithmetic mean (average) and geometric mean in baywide Chesapeake data.

Appendix B

Statistical Properties of Large Chlorophyll *a* Data Sets

Central Tendency vs Frequency of Exceeding a Threshold

Strong relationships are known to exist between the arithmetic mean (average) of chlorophyll *a* concentration and the percent exceedance of a chlorophyll threshold (e.g., Walker 1984). In Chesapeake Bay tidal waters, these relationships are found in all three types of chlorophyll *a* data: low-frequency shipboard data, DATAFLOW, and ConMon.

The top panel in **Figure B-1** shows the family of curves for seven exceedance thresholds: 10, 12, 15, 20, 22, 23, and 30 μg/liter. The data are from shipboard samples collected in the above-pycnocline layer once or twice monthly at fixed locations over the 30-year period between 1984 and 2013. Most samples were collected as part of the CBP monitoring program; all data are downloadable from the CBP Data Hub. The data were parsed into two seasons (spring, summer), five salinity zones (TF, OH, LoMH, HiMH, and PH), and six water quality categories (Best, Better, MBL, MPL, Poor, Worst;[7] new Secchi depth classification thresholds applied) for a total of 47 unique groupings (groups with less than 20 sampling events are not included). Examples of the relationships when these same data are grouped by a) station, b) CBP segment and year, and c) salinity zone and year are shown in Figure 9 of Buchanan (2014).

The middle panel in **Figure B-1** shows the family of eight exceedance curves generated from the spatially-rich data collected with the DATAFLOW method in the JMSPH segment in spring and summer between 2005 and 2013 (Egerton, pers. comm.). The chlorophyll *a* thresholds are: 10, 20, 30, 40, 50, 60, 70 and 80 μg/liter.

The bottom panel in **Figure B-1** was generated from the temporally-rich ConMon data collected at six fixed locations in Maryland nearshore waters of the Potomac River mainstem in summer (June – September), between 2004 and 2008. The figure shows the exceedance curve for a chlorophyll *a* threshold of 20 μg/liter. Data were provided by Maryland Department of Natural Resources.

Figures B-2 - **B-4** demonstrate that the close relationships hold regardless of which measure of central tendency (arithmetic mean, geometric mean, and median) is used on the x-axis. These graphs were generated from low-frequency monitoring data collected at fixed stations in Chesapeake Bay and its tidal tributaries between 1984 and 2013 in waters designated as "open waters" (greater than 2 m depth). The data are grouped by water quality categories (see Buchanan 2014 and 2015a for descriptions of the data and water quality categories; the revised Secchi depth thresholds from Buchanan 2015a were used in this analysis). Groups with fewer than 30 data points are excluded. In each figure, the upper graph shows the data points involved in generating the regression curve for one threshold (15 μg/liter), and the bottom graph shows the family of curves generated for multiple thresholds. The upper graphs give a sense of the variability in the results around the regression curve. The lower graphs give a sense of the consistency in the curves regardless of which measure of central tendency is used.

An interesting aspect to note in the upper graphs is the separation of points in Best and Better water quality categories from those in the other categories. Best and Better are environments with adequate water clarity for unstressed photosynthesis and limiting concentrations of both DIN ($\leq$0.07 mg/liter) and PO_4 ($\leq$0.007 mg/liter). Nutrients exert stronger controls on phytoplankton growth than light (desired condition). The MBL environment has adequate water clarity but excess concentrations of one or both

[7] Best and Better comprise the Reference category; Poor and Worst comprise the Degraded category.

nutrients. The remaining categories, generally considered degraded, have inadequate water clarity; those in the Poor and Worst categories have excess concentrations of both DIN and PO_4. A regression curve through the Best and Better categories (not shown) would be lower and flatter than the overall regression curve (shown). Similarly, in **Figures B-3** and **B-4** (where the measure of central tendency is the median and geometric mean, respectively), a regression curve through the Poor and Worst categories (not shown) falls above the overall regression curve. This result is an indication of the expected, moderating influence of nutrient concentration on chlorophyll *a* distributions and threshold exceedance rates.

In **Figures B-5 – B-7**, the same data are grouped by station with no regard for water quality category. The salinity assigned to the station's segment is indicated. The upper graphs show the relationships between exceedance rates for a threshold criterion of 15 µg/liter and the arithmetic mean, geometric mean, and median. The lower graphs show the exceedance rates for a threshold criterion of 23 µg/liter. Stations with fewer than 50 data points were removed.

The use of 3rd order polynomial regressions was for convenience here and other regression algorithms—such as LOWESS regressions or the algorithms discussed in Walker (1984)—may fit the Chesapeake Bay relationships better.

Central Tendency vs. an Upper Limit

Strong relationships are also found in chlorophyll *a* data sets between measures of central tendency and the distribution of chlorophyll *a* values around the central tendency. The relationships make it relatively easy to calculate the likely magnitude of values associated with a given mean. Examples are shown in **Figures B-8 – B-11**, with the same data used in **Figure B-1** (upper panel) and **Figure B-2 – B-7**. Stations with fewer than 50 data points are excluded to minimize outliers caused by too few samples. The 90th percentile of each station's data is treated as the upper limit (although other percentiles are just as effective). Each station's mean is plotted against its upper limit. The relationships are fairly tight, with linear regression r^2's between 0.75 and 0.97.

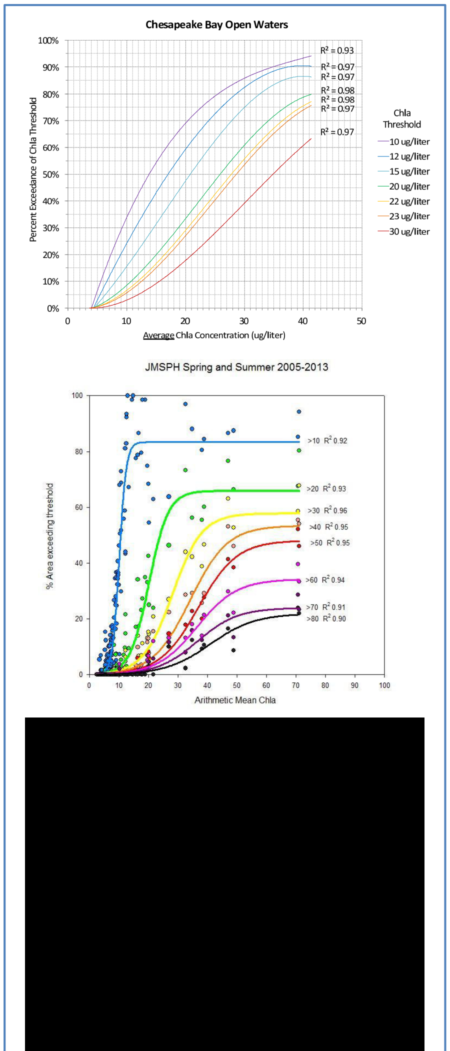

Figure B-1. Top: exceedance curves for multiple chlorophyll *a* thresholds, data from monthly or twice monthly spring and summer baywide shipboard data 1984-2013. **Middle**: exceedance curves for multiple thresholds, from summer James River DATAFLOW data (Egerton, pers. comm.). **Bottom:** exceedance curve for chlorophyll *a* ≥ 20 µg/liter, Potomac River ConMon data from Maryland flanks of mainstem (June-Sept, 2004-2008, see also Buchanan 2009).

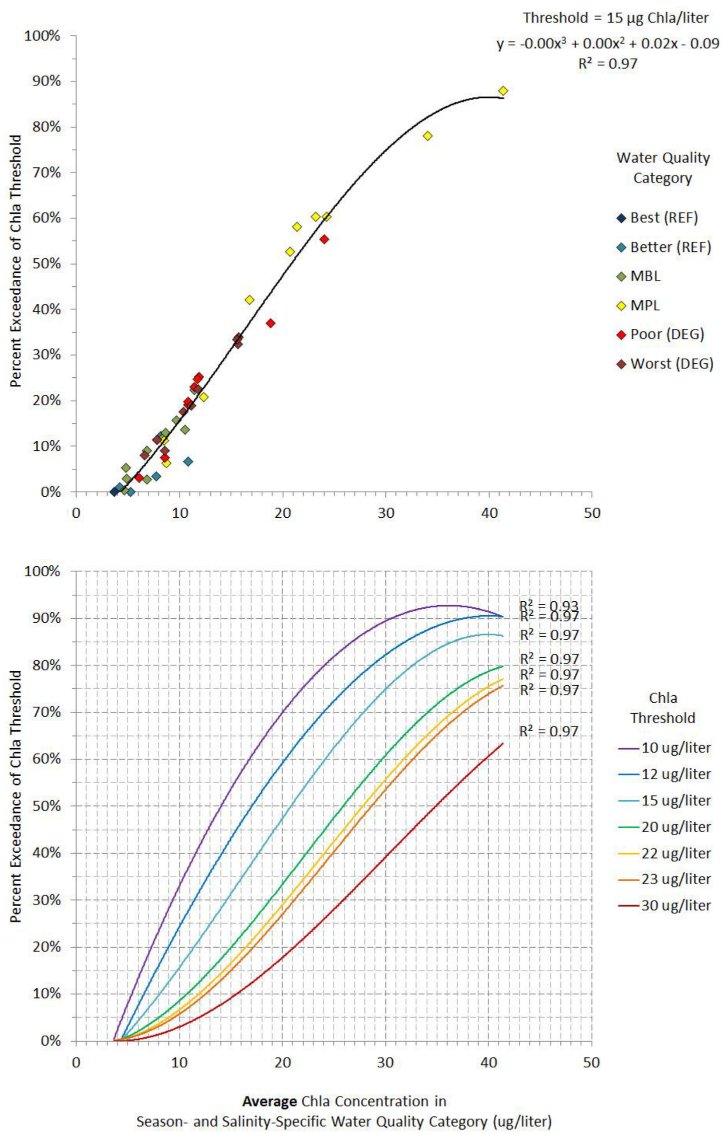

Figure B-2. Arithmetic mean *versus* rate of exceeding different thresholds. Data grouped by season- and salinity-based water quality categories (see text for details).

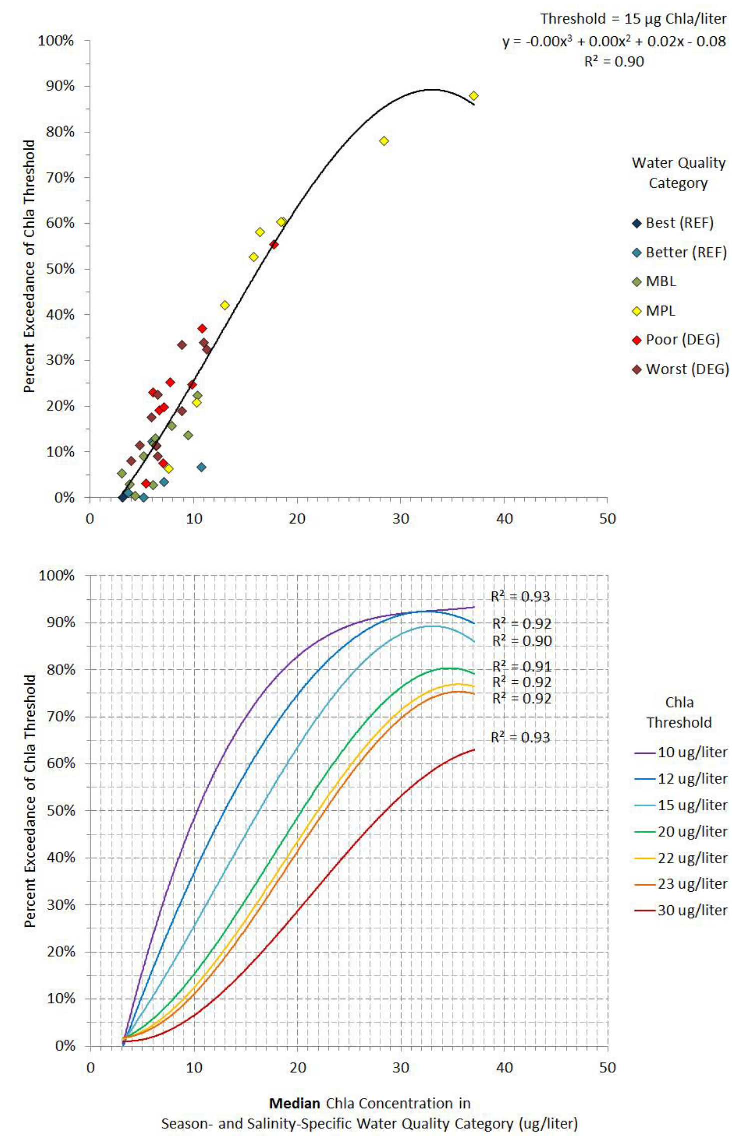

Figure B-3. Median *versus* rate of exceeding different thresholds. Data grouped by season- and salinity-based water quality categories (see text for details).

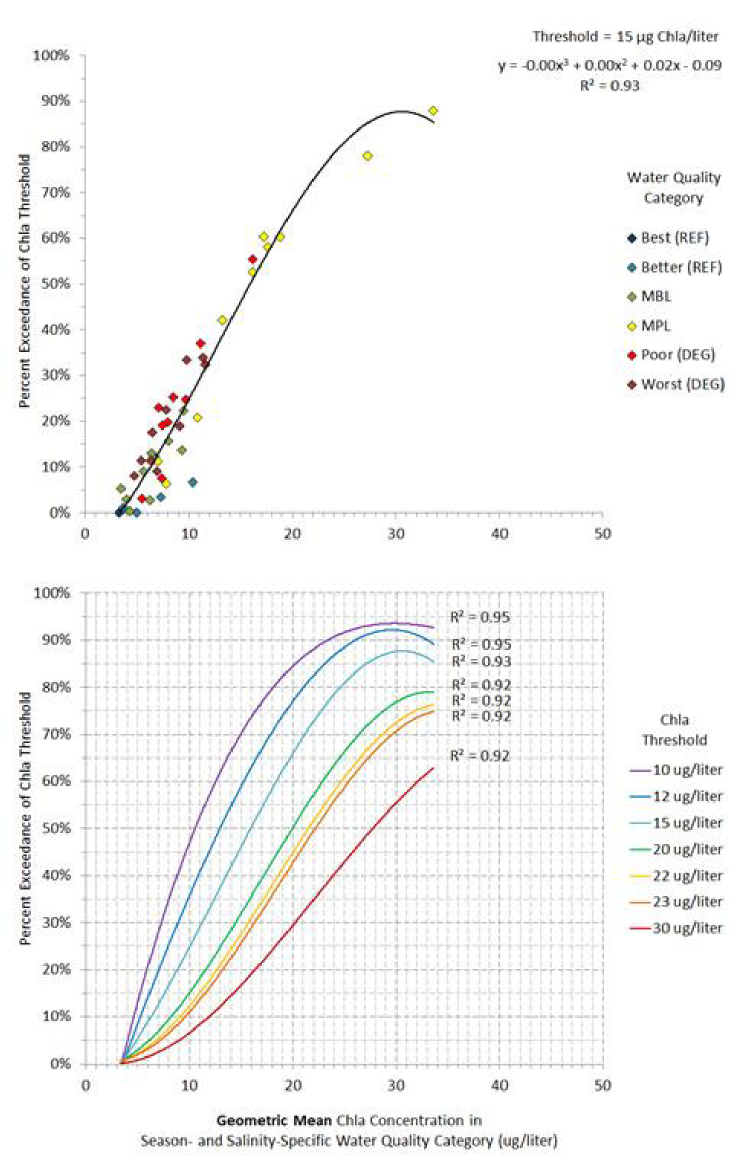

Figure B-4. Geometric mean *versus* rate of exceeding different thresholds. Data grouped by season- and salinity-based water quality categories (see text for details).

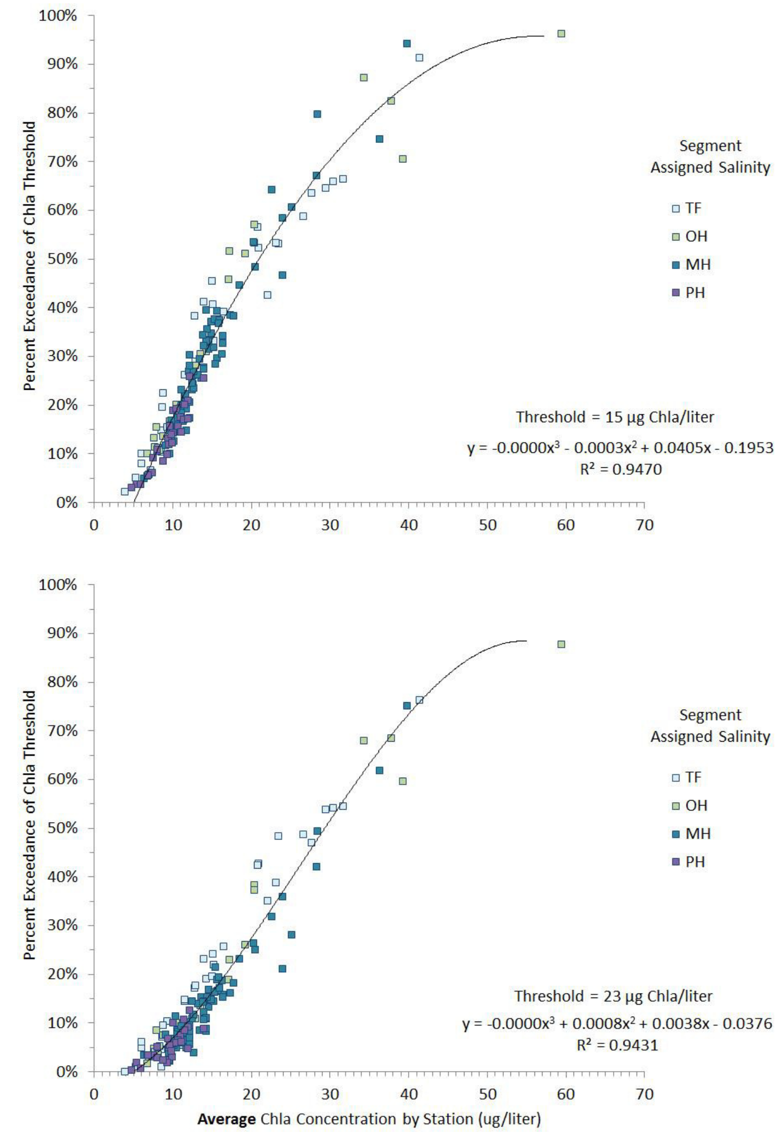

Figure B-5. Arithmetic mean *versus* rate of exceeding 15 (top) and 23 (bottom) µg/liter chlorophyll *a*. Data grouped by station irrespective of season. Segment's assigned salinity is indicated.

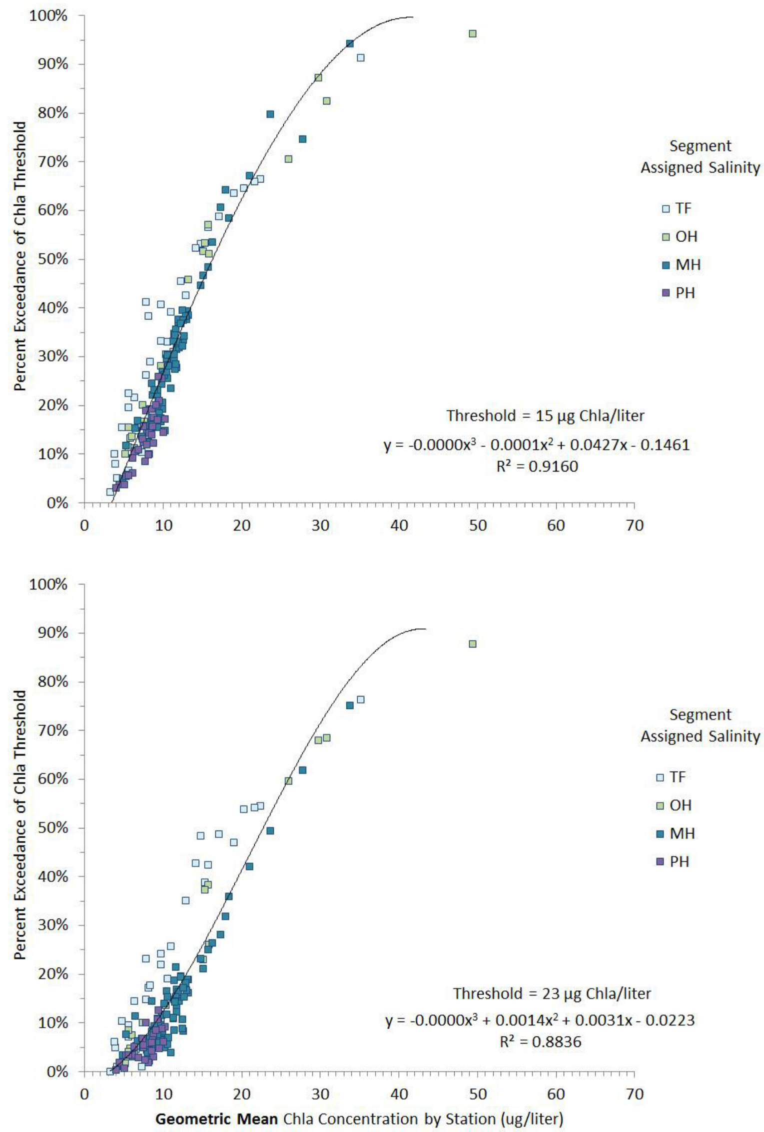

Figure B-6. Geometric mean *versus* rate of exceeding 15 (top) and 23 (bottom) µg/liter chlorophyll *a*. Data grouped by station irrespective of season. Segment's assigned salinity is indicated.

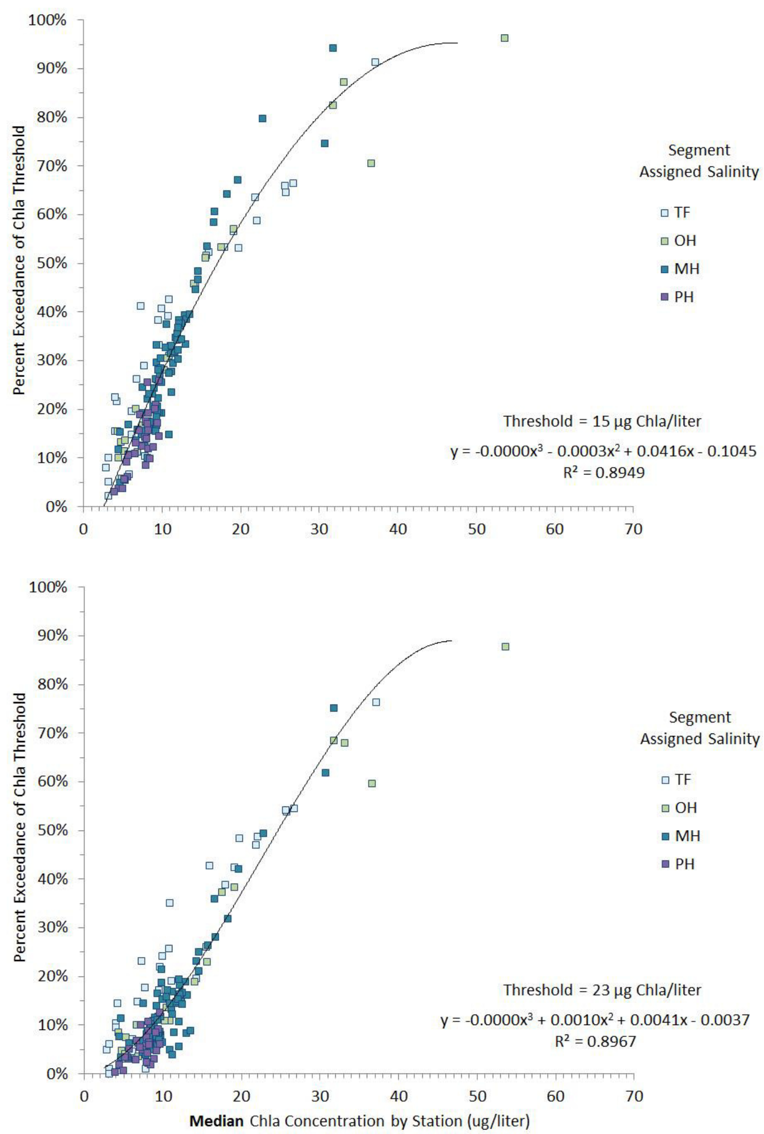

Figure B-7. Median *versus* rate of exceeding 15 (top) and 23 (bottom) µg/liter chlorophyll *a*. Data grouped by station irrespective of season. Segment's assigned salinity is indicated.

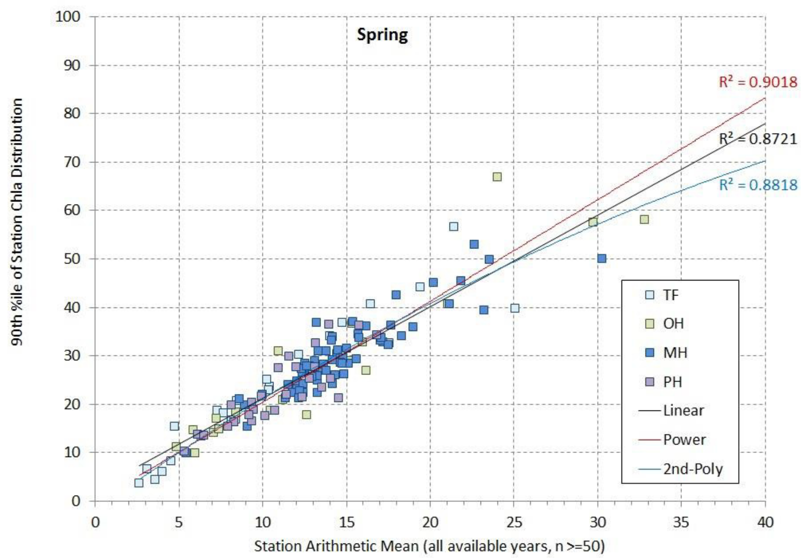

Figure B-8. Station arithmetic mean vs 90th percentile, Spring

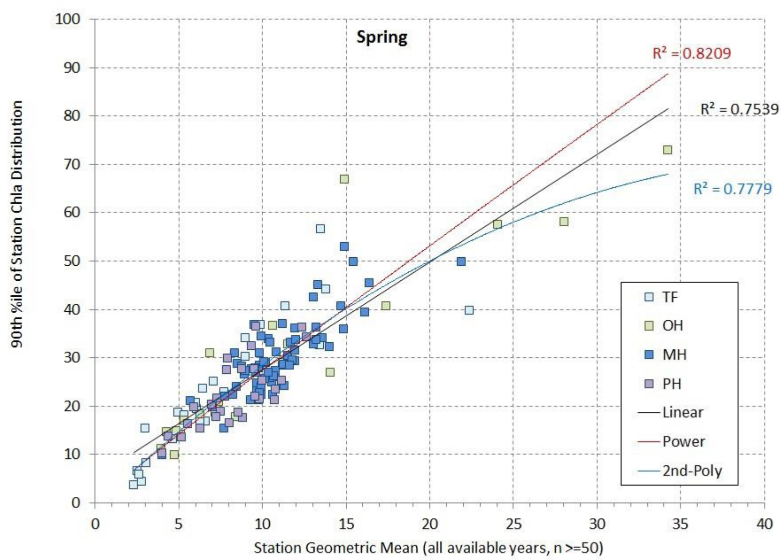

Figure B-9. Station geometric mean vs 90th percentile, Spring.

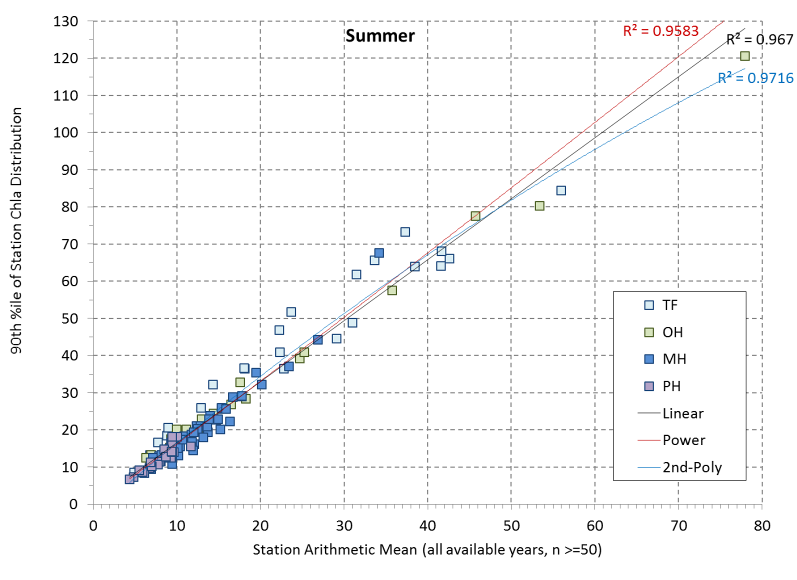

Figure B-10. Station arithmetic mean vs 90th percentile, Summer

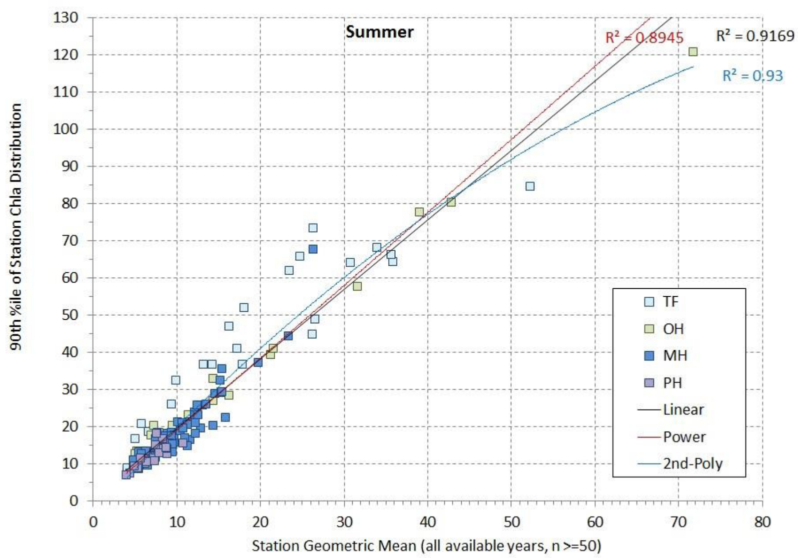

Figure B-11. Station geometric mean vs 90th percentile, Summer.

Appendix C

Interpretation and Application of Conditional Probability and Change Point Analysis Results

Conditional probability and change point analysis techniques can be good methods for discerning thresholds, but they should be used cautiously because some results can be strongly driven by environmental conditions unacknowledged in the analysis, such as water quality conditions. Spring Mesohaline PIBI scores are used here as one illustration (**Figure C-1**). All the individual Spring Mesohaline data points (Maryland and Virginia 1984-2010 data) were divided into two groups with opposing water quality conditions – Reference and Degraded ("Mixed" categories were excluded). In the figure, o's indicate samples from Reference conditions and +'s indicate samples from Degraded conditions. In the top panel, the lines are the conditional probabilities (right y-axis) for the two groups, separately and combined. Not surprisingly, the conditional probability line for the Reference group is much higher than that for the Degraded group and the line for the combined data falls in the middle. This middle line will move up or down depending on the proportions of Reference and Degraded samples in the analysis data set. A conditional probability of PIBI $\geq$ 2.67 starts high and drops sharply with increasing chlorophyll *a* in Reference conditions; it starts low and drops slowly with increasing chlorophyll *a* in Degraded conditions.

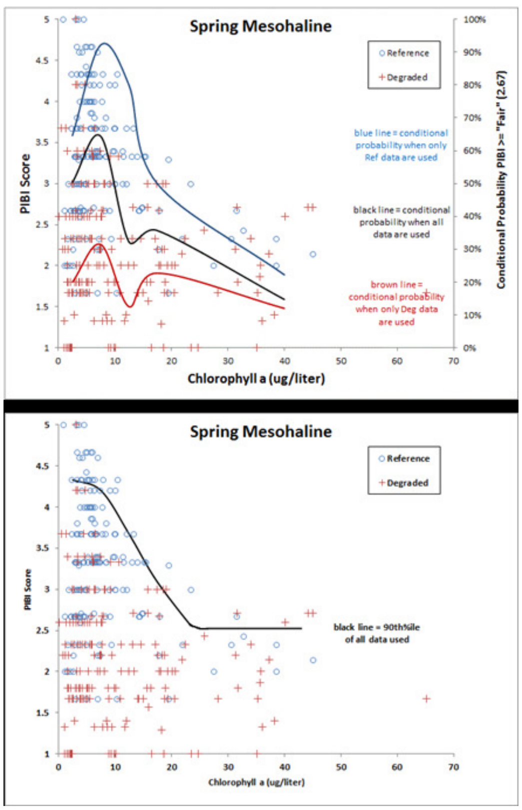

Figure C-1. Spring mesohaline PIBI scores in Reference and Degraded conditions. Conditional probabilities shown in upper panel. Quantile regression shown in bottom panel.

Quantile regression is an approach that gets around the effect of the underlying water quality effect for the most part. The bottom panel in **Figure C-1** shows the 90th%ile line on the combined data. It is creating an envelope (drawn free-form here) around the data using the 90th%iles. The quantile regression line is less affected by proportions of Reference and Degraded samples in the overall data set and it is often better at showing change points. Thresholds in the lower panel can be determined using logic, e.g., "the chl concentration where PIBI >= 2.67 begins to appear," or "the chl bin equidistant from the lower and upper breakpoints" (this would be in the 10-15 µg/liter range).

The effect of water quality condition on phytoplankton community status can also be seen when the PIBI and PTI scores are binned into chlorophyll *a* increments. The effect is clearly evident in high salinity Chesapeake waters (>10 ‰) where relative large numbers of REF+MBL water quality samples occur. In spring and summer (**Figure C-2** bottom panels), the majority of PIBI scores in Reference (dark blue) and MBL (light blue) conditions are $\geq$ 2.67, or Reference-like. Scores drop as chlorophyll *a* increases. PIBI scores in Mixed Poor Light (MPL) and Degraded (DEG) conditions are typically below 2.67, and scores drop slowly or not at all as chlorophyll *a* increases. The pattern in PIBI is less clear in low salinity Chesapeake waters (**Figure C-2**, upper panels), where Reference conditions are rare, nutrient concentrations in MBL conditions (surrogate for Reference) are relatively high and can stimulate growth, and all water quality categories, including DEG, show declining PIBI scores with increasing chlorophyll *a*. Baywide PIBI scores in MBL, however, are still higher than those in MPL and DEG.

The PTI scores in high salinity waters show an even sharper distinction than the PIBI between REF and DEG (**Figure C-3**). Most REF scores are greater than 50% while most DEG scores are less than 50%. Interesting, there does not appear to be a relationship with chlorophyll *a*. The consistent differences across the chlorophyll *a* increments indicate a strong underlying influence of water quality condition on phytoplankton composition. In two other examples, phytoplankton taxa richness and chlorophyll *a* cell content in high salinity waters (**Figure C-4, C-5**) shows distinct differences between REF and DEG categories and change with increasing chlorophyll *a* in one or more of the water quality categories.

In summary, caution should be used when applying change point analysis and conditional probability techniques to determine chlorophyll *a* thresholds. This is due to the underlying influence of water quality conditions on many phytoplankton parameters.

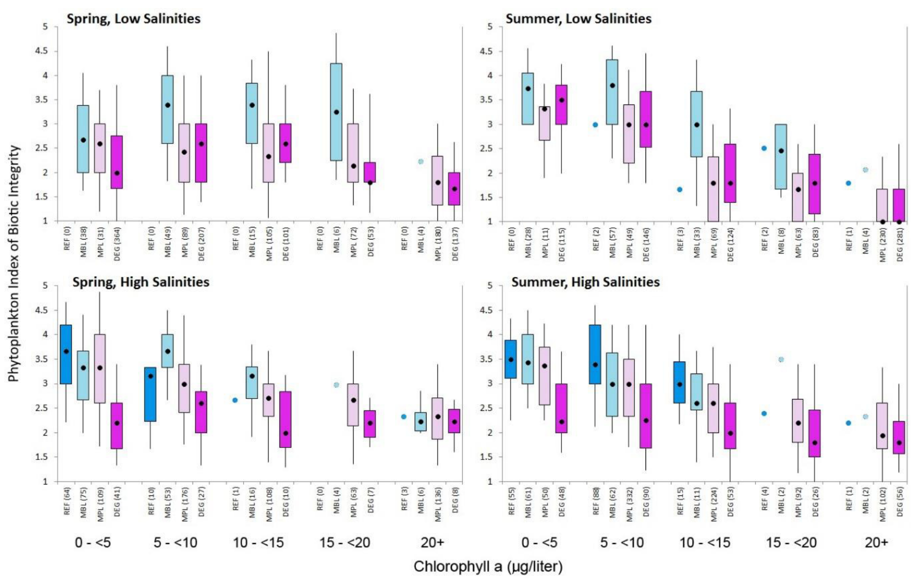

Figure C-2. Distribution of Phytoplankton Index of Biotic Integrity (PIBI) scores in chlorophyll *a* increments (baywide data set). Scores grouped by season, salinity (low, ≤10‰; high, >10‰), and water quality category (REF, MBL, MPL, DEG). Values in parentheses: sample sizes. Box-and-whiskers: 5th, 25th, 50th (median), 75th and 95th percentiles. Only the median is shown for groups with fewer than 5 samples. See text for details.

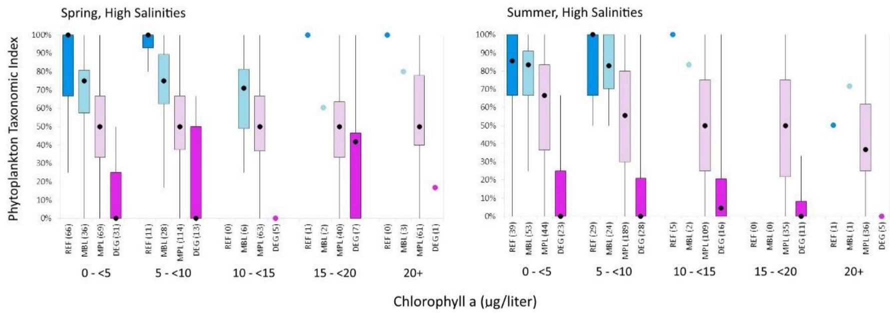

Figure C-3. Distribution of Phytoplankton Taxonomic Index (PTI) scores in chlorophyll *a* increments of high salinity waters (Virginia data only). See Figure C-2 heading for details.

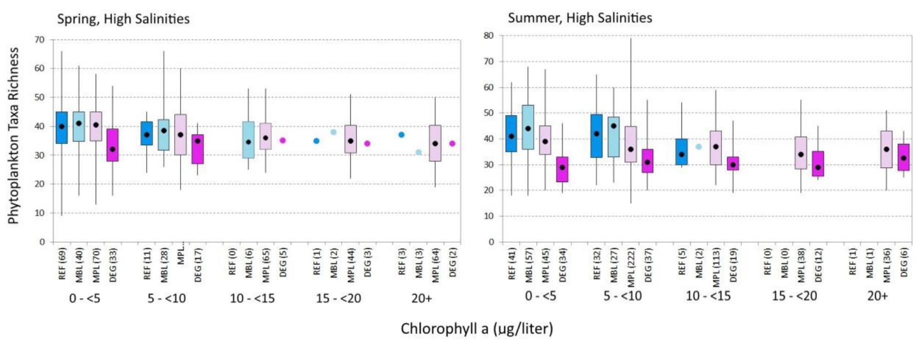

Figure C-4. Distribution of phytoplankton taxa richness values in chlorophyll *a* increments of high salinity waters (Virginia data only). See Figure C-2 heading for details.

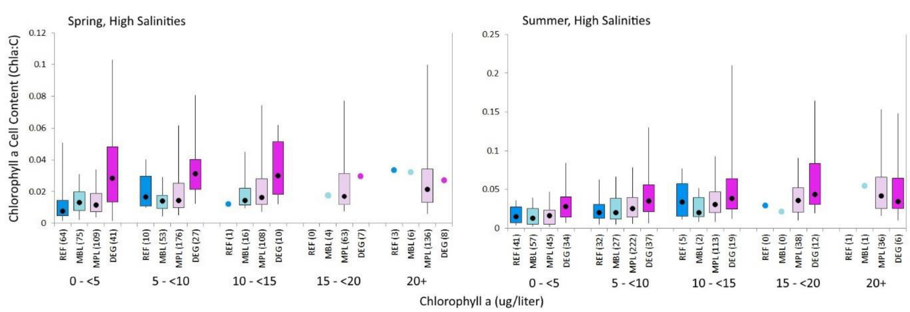

Figure C-5. Distribution of chlorophyll *a* cell content in chlorophyll *a* increments of high salinity waters (Virginia data only). See Figure C-2 heading for details.

Appendix D

Analytical Steps in Virginia's Chlorophyll Criteria Assessments

Virginia's first chlorophyll *a* criteria assessment procedures for the tidal James River were described in Chapter 5 of USEPA (2008). Assessments were done by segment using seasonal data from multiple fixed stations. DATAFLOW data were incorporated in assessments of the lower segments of the tidal James River. Impairment status is based on spatial and temporal frequency of exceedances in a three-year window using the CBP supported Cumulative Frequency Distribution (CFD) method (USEPA 2007a). The analytical steps for the assessor are:

1. Compile and QA/QC data set of chlorophyll *a* values for the 3-year assessment period.
2. Group data by date and segment.
3. Apply the CBP interpolation program and populate an assessment layer for each segment and sampling date with estimated chlorophyll *a* values (an assessment layer for chlorophyll *a* is the grid of surface water quality model cells in a segment).
4. For each interpolation cell, calculate a season-year arithmetic mean, or simple average, of chlorophyll *a* concentrations across all dates (**Figure D-1**). (*Changed in 2010 to geometric mean.*)
5. For each cell, determine if the **season-year average** of the cell violates the criteria.
6. Calculate the percent of all cells violating the criteria in the segment in a given year.
7. Determine the cumulative probability of the space violation rate (Weibull formula) for the three year assessment period (**Figure D-2**).
8. Construct a CFD (**Figure D-3**).
9. If any point of this CFD crosses the reference curve, the segment is deemed "impaired."

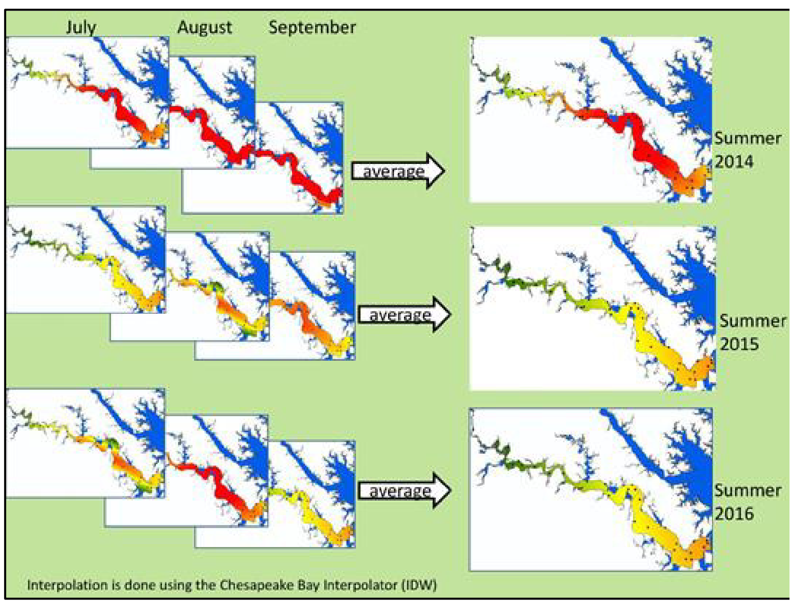

Figure D-1. Illustration of Virginia method of annual averaging from monthly chlorophyll *a* interpolations (from T. Robertson 2014).

Season-Year (ex. JMSTF1)	Space Violation Rate (Hypothetical)	Rank	Cumulative Probability Rank / (n+1)
Summer 2014	67%	1	25%
Summer 2016	10%	2	50%
Summer 2015	0%	3	75%

Figure D-2. Determine the cumulative probability (Weibull formula) for the three year assessment period (from T. Robertson 2014). Note that the cumulative probability is derived from just 3 points.

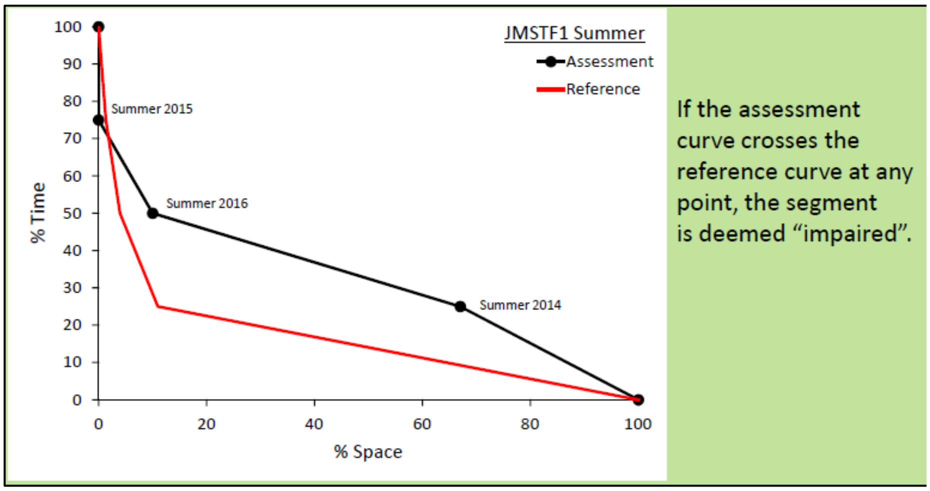

Figure D-3. Construct a CFD from the **three** cumulative probabilities (from T. Robertson 2014).

The Virginia methodology described above has evolved since it was first implemented. For example, USEPA (2010, Chapter 4) recently changed the Virginia assessment methodology so it now is to use the seasonal **geometric mean** instead of the seasonal arithmetic mean.

The Effect of Assessment Reference Curve on Criteria Protectiveness

In lieu of biology-based curves, EPA (2007a) recommends using a 10% hyperbolic curve as a default assessment reference curve when applying the Cumulative Frequency Distribution (CFD) procedure and determining criteria exceedances. The default curve shows the allowable criteria exceedances (10%) over space (x-axis) and time (y-axis). It provides equal weight to exceedances occurring in either time or space, and is consistent with past EPA national guidance on allowable exceedances.

While there is no specific theoretical basis for the default 10% hyperbolic curve, a recent data analysis gives its use some validity with respect to chlorophyll *a* in Chesapeake Bay (Buchanan 2014). A CFD assessment approach was used to analyze the baywide subset of REF+MBL data. The season- and salinity-specific upper percentiles (i.e., 90th and 95th percentiles) of this reference data set were applied as exceedance thresholds to the same data. The resulting CFD curves closely bracket the default 10% hyperbolic curve in the two seasons and four salinity groups (**Figure D-4**). The exceedances tended to

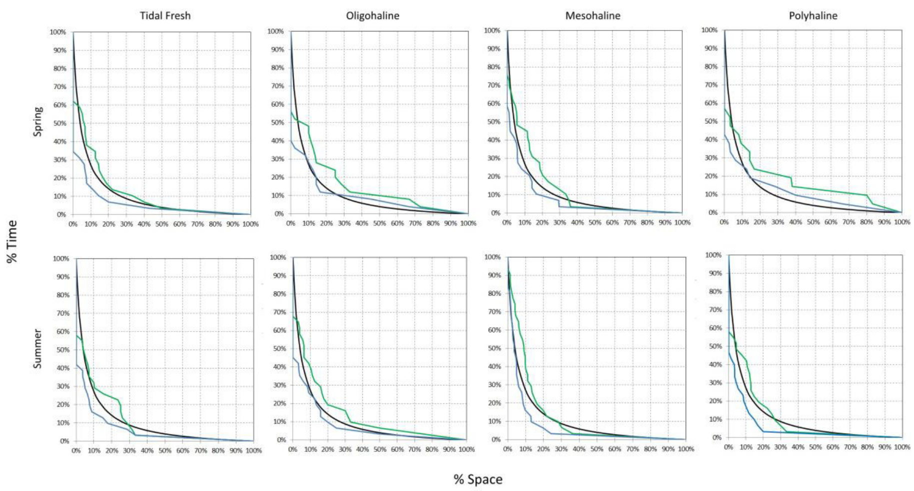

Figure D-4. Biology-based reference curves for chlorophyll *a* using a cumulative frequency distribution (CFD) assessment procedure and **upper percentiles** of the reference populations as exceedance thresholds. Data are all 1984 - 2013 baywide open water samples associated with REF+MBL conditions. Black line is the default 10% hyperbolic reference curve recommended by USEPA (2007a). Green and blue lines are the CFD curves generated when the 90th and 95th percentiles, respectively, of chlorophyll *a* concentrations in REF+MBL phytoplankton populations are used as exceedance thresholds. REF+MBL conditions that occur for entire seasons in an individual segment are currently non-existent in Chesapeake Bay, so the USEPA (2007a) time-vs-space basis for calculating the CFD curves was modified from month-segment to year-salinity zone in order to obtain adequate sample sizes. From Buchanan (2014).

distribute over time and space along the 10% hyperbolic curve, with small areas exceeding the percentiles occasionally and large areas exceeding the percentiles very rarely. When the numeric values of the tidal James River chlorophyll *a* criteria are applied as upper limits instead of as geometric means to the same phytoplankton reference population, four of the ten season- and segment-specific curves fall fairly close to the 10% hyperbolic curve and biology-based reference curves in Figure D-4: spring TF up (10), spring TF low (15), summer TF low (23), and summer OH (22). The numeric values of these four criteria (parentheses) approximate the 90th – 95th percentiles of their respective reference populations. Criteria whose numeric values are higher than the 90th – 95th percentiles of their reference population generate reference-based curves that fall below and to the left of the 10% hyperbolic curve. Those whose numeric values are lower than the 90th – 95th percentiles are above and to the right of the curve.

This exercise demonstrates that CFDs generated from exceedances of the upper percentiles of chlorophyll *a* values found in Chesapeake phytoplankton reference populations will approximate the 10% hyperbolic curve. The curve appears to be a suitable default reference assessment curve when 90% or more of the data are expected to fall below a criterion at attainment and only 10% are allowed to exceed it. It is a reasonable representation of the natural spatial and temporal extent of algal blooms in Chesapeake Bay under reference conditions. James River criteria, however, are expressed as geometric means and not upper thresholds. By nature of the fact that the geometric mean is a measure of central tendency calculated from log-transformed values, almost half the values comprising the mean can be expected on average to exceed the criterion when it reaches attainment. Therefore, a CFD assessment curve reflecting attainment of a geometric mean criterion should differ from the 10% hyperbolic curve and allow almost half of the data to exceed the criterion at attainment.

This is indeed the result when geometric means instead of upper percentiles of the phytoplankton reference data are applied as exceedance thresholds. On average, about 46% of individual chlorophyll *a* values are above the season- and salinity-specific geometric means found in the combined REF+MBL data; 54% are below. CFD assessment curves derived from these data fall close to or somewhat below a 50% line crossing the %time-%space assessment graph (**Figure D-5**).

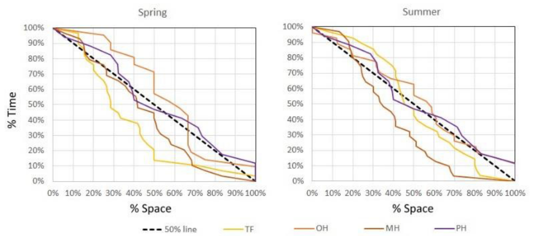

Figure D-5. Biology-based assessment reference curves for chlorophyll *a* using a cumulative frequency distribution (CFD) assessment approach and the **geometric means** of chlorophyll *a* in phytoplankton reference populations (combined REF and MBL) as exceedance thresholds. The geometric means used in to represent reference populations in these graphs are: 3.4 (Spr TF), 9.5 (Spr OH), 7.35 (Spr MH), 3.8 (Spr PH), 6.4 (Sum TF), 5.7 (Sum OH), 8.1 (Sum MH), and 4.3 (Sum PH) µg/liter. See text and Figure D-4 for details.

As a very rough guide, the 50% line appears to be the appropriate location for a default assessment reference curve on the %time-%space assessment graph when any criteria are expressed as measures of central tendency. CFD curves that are above and to the right of the 50% line will indicate geometric means that frequently exceed the criteria and populations of measurements that are out of attainment. Curves below and to the left of the 50% line will indicate varying degrees of criteria over-achievement, where geometric means are lower than levels needed to attain the 50% line. Curves passing through the lower left corner signify 100% attainment with no criteria exceedances.

As discussed above, VADEQ determines attainment of the James River chlorophyll *a* criteria by comparing CFD assessment curves to a default assessment reference curve, which is the 10% hyperbolic curve. Since the criteria are geometric means and not upper thresholds, attainment of the default assessment reference curve effectively requires over-achievement of the criteria. Geometric means must be substantially lower than those comprising the CFD assessment curves along the 50% line in order to meet the default 10% curve. In VADEQ assessments, the stringency needed to meet the 10% curve is countered by the fact that the numeric values of the James River chlorophyll *a* criteria are higher than the geometric means of phytoplankton reference populations (**Table 2**). Attainment of the 10% curve by the current James River criteria expressed as geometric means can be to varying degrees protective of desirable phytoplankton populations. This paradox is illustrated in **Figure D-6**.

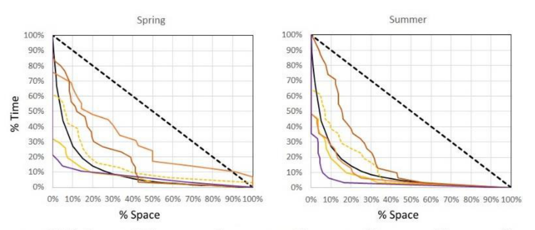

Figure D-6. Biology-based assessment reference curves for chlorophyll *a* using a cumulative frequency distribution (CFD) assessment approach and the **James River chlorophyll *a* criteria** as exceedance thresholds. See text and Figure D-4 for details.

The 50% (dashed black) line in **Figure D-6** indicates roughly where the CFD assessment curves will occur when James River criteria first are attained. Since the numeric values of the criteria are all higher than the geometric means of phytoplankton reference populations, attainment of this 50% line will not represent protection of desirable reference phytoplankton in any season or salinity. If water quality conditions continue to improve, the proportions of geometric means failing the criteria will decrease below 50%, and the CFD assessment curves will shift toward the lower left corner. Distributions of chlorophyll *a* values within these phytoplankton populations will be lower and begin to resemble those in the reference populations. Fewer and fewer will exceed the James River criteria.

When phytoplankton reference populations are actually achieved, CFD assessment curves derived with the James River chlorophyll *a* criteria will resemble the curves shown in color in **Figure D-6**. They can be separated into two groups: those found below and to the left of the 10% hyperbolic curve (black solid line) and those found between that curve and the 50% line (black dashed line). CFD curves for the first group (Spring TF low, Spring PH, Summer TF low, Summer OH, and Summer PH) experience fewer than 10% of geometric means exceeding the criteria. The numeric values of the criteria in these five season-salinities tend to be much higher (~3.5X) than the corresponding geometric means in reference populations. Thus, geometric means in the reference population have a very low probability of exceeding these James River criteria and the CFD assessment curves fall closest to the origin (0,0). The second group of criteria (Spring TF up, Spring OH, Spring MH, Summer TF up, and Summer MH) have numeric values that are only ~2X higher than the corresponding geometric means in reference populations. The probability of reference population geometric means exceeding the James River criteria in these groups is also low, but not as low as in the first group.

The degree to which the current criteria's CFD curves deviate from the 50% line in **Figure D-6**, when the criteria are applied to reference populations, indicates their level of protectiveness of reference populations. The first group of criteria, with relatively high numeric values and CFD curves falling behind the 10% hyperbolic curve, are less protective because the 10% curve will be attained before reference populations are achieved. The spring TF low, spring PH and summer PH criteria are clearly under-protective; CFD curves for summer TF low and summer OH are close enough to the 10% curve that the criteria could be considered somewhat protective. Criteria of the second group are actually protective of the reference populations *before* their CFD curves achieve the 10% curve. Attaining the 10% curve requires over-achievement of the criteria. The Spring OH, spring MH, summer MH, and summer TF up criteria are clearly over-protective; the CFD curve for spring TF up is close enough to the 10% curve that it could be considered somewhat protective. **Table D-1** summarizes these results.

Given that REF+MBL conditions do not occur yet over entire seasons in Chesapeake assessment segments and a time-for-space exchange was used to calculate CFD assessment curves for phytoplankton reference populations, the results in this section should be viewed as hypothetical. The fact remains, however, that VADEQ significantly increases the protectiveness of its current James River criteria by using the 10% hyperbolic curve as the default assessment reference curve in its current assessment methodology. Fewer than 10% of seasonal *geometric means* are allowed to exceed the numeric values of the criteria in a segment over an assessment period.

Table D-1. Possible protection of phytoplankton reference populations by the current James River chlorophyll *a* criteria when expressed as geometric means *and* evaluated against the 10% hyperbolic curve as the default assessment reference. Geometric means and upper percentiles of chlorophyll *a* in phytoplankton reference populations (REF+MBL) are indicated. **Note** that numeric values of the "somewhat protective" criteria fall between the 90th and 95th percentiles of the REF+MBL populations. Numeric values of the "over-protective" criteria are below these percentiles; numeric values of the "under-protective" criteria are above these percentiles.

Season/Segment		REF+MBL geomean	REF+MBL 90th / 95th	Criteria	Possible protectiveness
Spring	TF up	3.4	10.4 / 13.5	10	Somewhat protective
	TF low	3.4	10.4 / 13.5	15	Under-protective
	OH	9.5	22.5 / 28.6	15	Over-protective
	MH	7.35	15.5 / 22.5	12	Over-protective
	PH	3.8	7.9 / 10.1	12	Under-protective
Summer	TF up	6.4	16.9 / 24.2	15	Over-protective
	TF low	6.4	16.9 / 24.2	23	Somewhat Protective
	OH	5.7	17.2 / 23.8	22	Somewhat Protective
	MH	8.1	11.9 / 14.2	10	Over-protective
	PH	4.3	7.4 / 8.7	10	Under-protective